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Catalytic Antibodies

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Glossary

Abzyme An alternative name for a catalytic antibody (derived from Antibody-enzyme).

Affinity labelling A method of identifying peptides located in the antigen binding site. The antibody is treated with a *hapten* which binds to the binding site and to proximal amino acid residues. Upon hydrolysis of the antibody, peptide fragments bound to the hapten are separated and identified.

Antibodies Proteins of the immunoglobulin superfamily, carrying *antigen*-binding sites that bind noncovalently to the corresponding *epitope*. They are produced by B lymphocytes (*B cells*) and are secreted from plasma cells in response to antigen stimulation.

Antigen A molecule, usually peptide, protein or polysaccharide, that elicits an immune response when introduced into the tissues of an animal.

B cells (also known as B lymphocytes) Derived from the bone marrow, where they differentiate into antibody-forming plasma cells and B memory cells, these cells are mediators of humoral immunity in response to *antigens*.

Bait and switch A strategy whereby the charge–charge complementarity between antibody and hapten is exploited. By immunizing with haptens containing charges directed at key points of the reaction transition state, complementary charged residues are induced in the active site which are then used in catalysis of the substrate.

BSA Bovine serum albumin, derived from cattle serum and used as a *carrier* protein.

Carrier protein Macromolecule to which a hapten is conjugated, thereby enabling the hapten to stimulate the immune response.

catELISA Similar to an *ELISA*, except that the assay detects catalysis as opposed to simple binding between hapten and antibody. The substrate for a reaction is bound to the surface of the microtitre plate, and putative catalytic *antibodies* are applied. Any product molecules formed are then detected by the addition of anti-product antibodies, usually in the form of a polyclonal mixture raised in rabbits. The ELISA is then completed in the usual way, with an anti-rabbit "second antibody" conjugated to an enzyme, and the formation of coloured product upon addition of the substrate for this enzyme. The intensity of this colour is then indicative of the amount of product formed, and thus catalytic antibodies are selected directly.

Conjugate In immunological terms this usually refers to the product obtained from the covalent coupling of a protein (e.g. a *carrier* protein) with a hapten, with a label such as fluorescein or with an enzyme.

Conjugation The process of covalently bonding (multiple) copies of a hapten to a carrier protein, usually by means of a linker to distance the hapten from the surface of the carrier protein by a chain of about six atoms.

ELISA (Enzyme-linked immunosorbent assay) An immunoassay in which *antibody* or *antigen* is detected. To detect antibody, antigen is first adsorbed onto the surface of microtitre plates, after which the test sample is applied. Any unbound (non-antigen-specific) material is washed away, and remaining antibody–antigen complexes are detected by an antiimmunoglobulin *conjugated* to an enzyme. When the substrate for this enzyme is applied, a coloured product is formed which can be measured spectrophotometrically. The intensity of the coloured product is proportional to the concentration of antibody bound.

Enhancement ratio, ER Quantified as k_{cat}/k_{uncat}, is used to express the catalytic power of a biocatalyst. It is a comparison between the catalysed reaction occurring at its optimal rate and the background rate.

Entropic trap A strategy aimed at improving the efficiency of catalytic *antibodies*, via the incorporation of a molecular constraint into the transition state analogue that gives the hapten a higher energy conformation than that of the reaction product.

Epitope The region of an *antigen* to which *antibody* binds specifically. This is also known as the antigenic determinant.

Fab′ The fragment obtained by pepsin digestion of *immunoglobulins*, followed by reduction of the interchain disulfide bond between the two heavy chains at the hinge region. The resulting fragment is similar to a Fab fragment in that it can bind with *antigen* univalently, but it has the extra hinge region of the heavy chain.

Fab The fragment obtained by papain hydrolysis of *immunoglobulins*. The fragment has a molecular weight of ~45 kDa and consists of one light chain linked to the N-terminal half of its corresponding heavy chain. A Fab contains one *antigen* binding site (as opposed to bivalent *antibodies*), and can combine with antigen as a univalent antibody.

Hapten Substance that can interact with antibody but cannot elicit an immune response unless it is conjugated to a *carrier protein* before its introduction into the tissues of an animal. Haptens are mostly small molecules of less than 1 kDa. For the generation of a catalytic antibody, a *TSA* (*q.v.*) is attached to a spacer molecule to give a hapten of which multiple copies can be linked to a carrier protein (*q.v.*).

Hybridoma Cell produced by the fusion of antibody-producing plasma cells with myeloma/carcinoma cells. The resultant hybrids have then the capacity to produce antibody (as determined by the properties of the plasma cells), and can be grown in continuous culture indefinitely owing to the immortality of the myeloma fusion partner. This technique enabled the first continuous supply of *monoclonal antibodies* to be produced.

IgG The major immunoglobulin in human serum. There are four subclasses of IgG; IgG1, IgG2, IgG3 and IgG4, but this number varies in different species. All are able to cross the placenta, and the first three subclasses fix complement by the classical pathway. The molecular mass of human IgG is 150 kDa and the normal serum concentration in man is 16 mg ml^{-1}.

Immunoglobulin Member of a family of proteins containing heavy and light

chains joined together by interchain disulfide bonds. The members are divided into classes and subclasses, with most mammals having five classes (IgM, IgG, IgA, IgD and IgE).

k_{cat} The rate constant for the formation of product from a particular substrate. k_{cat} is obtained by dividing the Michaelis–Menten parameter, V_{max}, by the total enzyme concentration. In real terms, the constant is a measure of how rapidly an enzyme can operate once its active site is occupied.

KLH Keyhole limpet haemocyanin, used for its excellent antigenic properties. It is used as a *carrier protein* in order to bestow immunogenicity in small haptens.

K_m The Michaelis–Menten constant, which is defined as the substrate concentration at which the biocatalyst is working at half its maximum rate (V_{max}). In practice, K_m gives a measure of the binding affinity between the substrate and biocatalyst; the smaller the value, the tighter the binding in the complex.

Library A collection of antibodies, usually Fab or scFv fragments, in the range of 10^6 to 10^{10} and displayed on the surface of bacteriophage whose DNA gene contains a DNA sequence capable of expression as the antibody protein. Thus, identification of a single member of the library by selection can be used to generate multiple copies of the phage and sizeable amounts of the antibody protein.

Monoclonal antibody, mAb Describes an antibody derived from a single clone of cells or a clonally obtained cell line. Its common use denotes an antibody secreted by a hybridoma cell line. Monoclonal *antibodies* are used very widely in the study of antigens, and as diagnostics.

Polyclonal antibodies Antibodies derived from a mixture of cells, hence containing various populations of antibodies with different amino acid sequences. They are of limited use in that they will not all bind to the same *epitopes* following immunization with a *hapten/carrier protein conjugate*. They are also difficult to purify and characterize, but have been used with success in the *catELISA* system.

Positive clones A phrase usually used to describe those hybridoma clones which bind reasonably to their respective hapten in an enzyme-linked immunosorbent assay, thereby eliminating non-specific *antibodies* raised to different *epitopes* of the hapten/carrier *conjugate*.

Residues General term for the unit of a polymer, that is the portion of a sugar, amino acid or nucleotide that is added as part of the polymer chain during polymerization.

Single-chain antibody (scFv) Comprises a V_L linked to a V_H chain via a polypeptide linker. It is thus a univalent functioning antibody containing both of the variable regions of the parent antibody.

Site-directed mutagenesis Induced change in the nucleotide sequence of DNA aimed at particular nucleotide residues, usually in order to test their function.

Somatic hypermutation Mutations occurring in the variable region genes of the light and heavy chains during the formation of memory B cells. Those B cells whose affinity is increased by such mutations are positively selected by interaction with *antigen*, and this leads to an increase in the average affinity of the *antibodies* produced.

Specificity constant Defined as k_{cat}/K_m. It is a pseudo-second-order rate constant which, in theory, would be the actual rate constant if formation of the enzyme–substrate complex were the rate-determining step.

TSA (Transition state analogue) Frequently a stable analogue of an unstable, high-energy reaction intermediate that is close to related energy barriers in a multi-step reaction.

1 Introduction

This review addresses most of the important advances that have occurred in the field of catalytic antibodies since the first reports a decade ago (Pollack *et al.*, 1986; Tramontano *et al.*, 1986). One of the most stimulating features of this subject is that it is not confined to a single scientific discipline. Therefore, although this article looks at catalytic antibodies and their activities from a physical organic chemistry viewpoint, it seeks to provide a self-contained review requiring only a rudimentary biochemical knowledge of antibody structure, function and production. Adequate details of these matters have been supplied, including a glossary of many of the immunological terms employed written in general chemical language; these are included to stimulate rather than discourage the reader. The survey does not seek to be fully comprehensive, but rather focuses on the more significant parts of a subject which, in a little over ten years, has achieved much more than most pundits expected from this scientific prodigy in its infancy. However, a fairly complete survey of the literature is presented in the form of an Appendix, which tabulates over 120 examples of reactions catalysed, the haptens employed, and the kinetic data reported.

ANTIBODIES AND THEIR BIOLOGICAL ROLE

The immune response provides one of the most important biological defence mechanisms for higher organisms. It depends on the rapid generation of structurally novel proteins that can identify and bind tightly to foreign substances of potential harm to the parent organism. This family of proteins are the immunoglobulins. In their simplest form, they are made up of two pairs of polypeptide chains of different length and interconnected by disulfide bridges. The two light and two identical heavy chains contain repeated

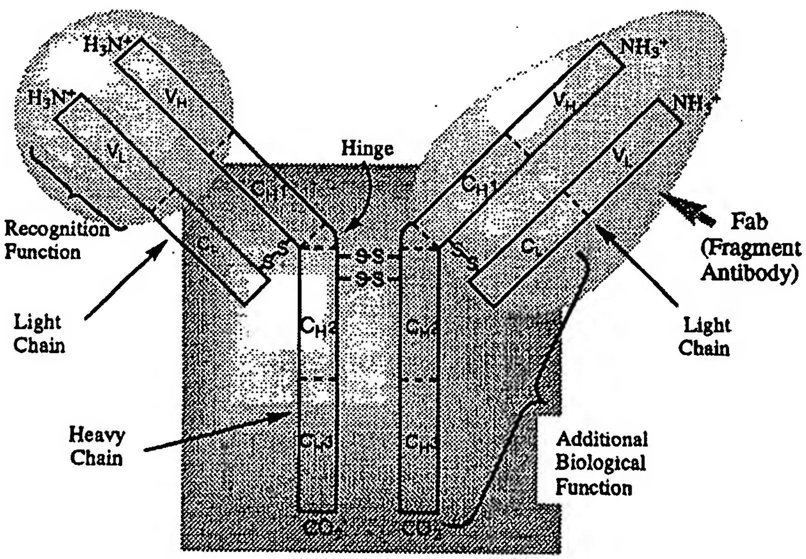


Fig. 1 Schematic structure of the peptide components of an IgG immunoglobulin showing the two light (L) and two heavy (H) polypeptide chains, the disulfide bridges connecting them (—S—S—); the four variable regions of the light (V_L) and heavy (V_H) chains, and the 8 "constant" regions of the light (C_L) and heavy (C_H^1, C_H^2, C_H^3) chains (shaded rectangle). Hypervariable regions that provide antigen recognition and binding are located within six polypeptide loops, three in the V_L and three in the V_H sections (shaded circle, top left). These can be excised by proteolytic cleavage to give a fragment antibody, Fab (shaded lobe, top right).

homologous sequences of about 110 amino acids which fold individually into similar structural domains, essentially a bilayer of antiparallel β-pleated sheets. This leads to an IgG immunoglobulin molecule whose core structure is formed from 12 similar structural domains: 8 from the two heavy chains and 4 from the two light chains (Fig. 1) (Burton, 1990).

By contrast, the N-terminal regions of antibody light and heavy chains vary greatly in the sequence and number of their constituent amino acids and thereby provide binding regions of enormous diversity, approaching 10^{10} in number for higher mammals. The remarkable property of the immune system is its ability to respond to single or multiple alien species by rapid diversification of the sequences of these hypervariable regions through mutation, gene splicing, and RNA splicing. This generates a vast number of different antibodies which are selectively amplified in favour of those with the strongest affinity for the alien species.

THE QUEST FOR A NEW CLASS OF BIOCATALYST

In the mid-1940s Linus Pauling clearly stated the theory that enzymes work by their complementarity to the transition state for the reaction to be catalysed (Pauling, 1948). This concept was, with hindsight, a logical extension of the then relatively new transition state theory that had been developed to explain chemical catalysis (Evans and Polanyi, 1935; Eyring, 1935). Its fundamentals support the proposition that the rate of a reaction is related to the difference in Gibbs free energy (ΔG°) between the ground state of reactant(s) and the transition state for the given reaction. For catalysis to occur, either the energy of the transition state has to be lowered (transition state stabilization) or the energy of the substrate has to be elevated (substrate destabilization). Pauling applied this to enzyme catalysis by stating that an enzyme preferentially binds to and hence stabilizes the transition state for a reaction over ground state of substrate(s) (Fig. 2). This has become a classical dogma in enzymology and is widely used to explain the way in which such biocatalysts are able to enhance specific processes with rate accelerations of up to 10^{17} over background (Albery and Knowles, 1976, 1977; Albery, 1993 for a recent review).

Pauling apparently did not bring ideas about antibodies into his concept of enzyme catalysis, though there is a tantalizing photograph in the volume of Pauling's Silliman lectures at Yale in 1947 which shows on a single blackboard cartoon both an energy profile diagram for the lowering of a transition state energy profile and also reference to an immunoglobulin (Pauling, 1947). And so it fell to Bill Jencks in his unsurpassed 1969 work on catalysis (Jencks, 1969)

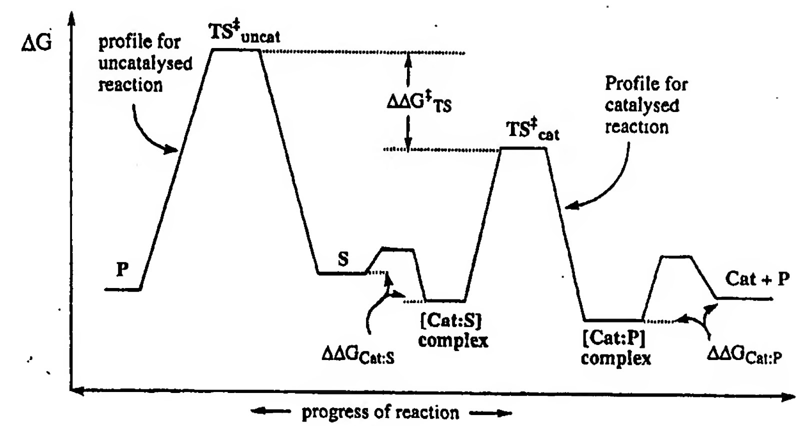


Fig. 2 Catalysis is achieved by lowering the free energy of activation for a process, i.e. a catalyst must bind more strongly to the transition state ($TS^{\ddagger}$) of the reaction than to either reactants or products. Thus: $\Delta\Delta G^{\ddagger} \gg \Delta\Delta G_{Cat:S}$ and $\Delta\Delta G_{Cat:P}$

to bring together the opportunity for synthesis of an enzyme using antibodies positively engineered in the immune system:

> "One way to do this [i.e. synthesize an enzyme] is to prepare an antibody to a haptenic group which resembles the transition state of a given reaction."[1]

The practical achievement of this goal was held up for 18 years, primarily because of the great difficulty in isolation and purification of single-species proteins from the immune repertoire. During that time, many attempts to elicit catalysis by inhomogeneous (i.e. polyclonal) mixtures of antibodies were made and failed (e.g. Raso and Stollar, 1975; Summers, 1983). The problem was resolved in 1976 by Köhler and Milstein's development of hybridoma technology, which has made it possible today both to screen rapidly the "complete" immune repertoire and to produce *in vitro* relatively large amounts of one specific monoclonal antibody species (Köhler and Milstein, 1975; Köhler *et al.*, 1976).

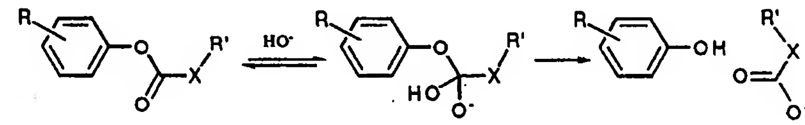
While transition states have been discussed in terms of their free energies, there have been relatively few attempts to describe their structure at atomic resolution for most catalysed reactions. Transition states are high-energy species, often involving incompletely formed bonds, and this makes their specification very difficult. In some cases these transient species have been studied using laser femtochemistry (Zewail and Bernstein, 1988), and predictions of some of their geometries have been made using molecular orbital calculations (Houk *et al.*, 1995). Intermediates along the reaction coordinate are also often of very short lifetime, though some of their structures have been studied under stabilizing conditions while their existence and general nature can often be established using spectroscopic techniques or trapping experiments (March, 1992b).

The Hammond postulate predicts that if a high-energy intermediate occurs along a reaction pathway, it will resemble the transition state nearest to it in energy (Hammond, 1955). Conversely, if the transition state is flanked by two such intermediates, the one of higher energy will provide a closer approximation to the transition state structure. This assumption provides a strong basis for the use of mimics of unstable reaction intermediates as transition state analogues (Bartlett and Lamden, 1986; Alberg *et al.*, 1992).

FIRST EXAMPLES OF CATALYTIC ANTIBODIES

In 1986, Richard Lerner and Peter Schultz independently reported antibody catalysis of the hydrolysis of aryl esters and of carbonates, respectively (Pollack *et al.*, 1986; Tramontano *et al.*, 1986). Reactions of this type are well

[1] Jencks apparently was not aware of Pauling's idea when he made this statement (Jencks, 1997, personal communication).



[1] Reactant [2] Tetrahedral Intermediate Products

Fig. 3 The hydrolysis of an aryl ester [1] ($X = CH_2$) or a carbonate [1] ($X = O$) proceeds through a tetrahedral intermediate [2] which is a close model of the transition state for the reaction. It differs substantially in geometry and charge from both reactants and products.

known to involve the formation and breakdown of an unstable tetrahedral intermediate, and so this can be deemed to be closely related to the transition state ($TS^‡$) of the reaction (Fig. 3).

Transition states of this tetrahedral nature have now been mimicked effectively by a range of stable analogues, including phosphonic acids, phosphonate esters, α-difluoroketones, and hydroxymethylene functional groups (Jacobs, 1991). Lerner's group elicited antibodies to a tetrahedral anionic phosphonate hapten [3] (Appendix entry 2.9)[2] whilst Schultz's group isolated a protein with high affinity for *p*-nitrophenyl cholyl phosphate [4] (Fig. 4) (Appendix entry 3.2).

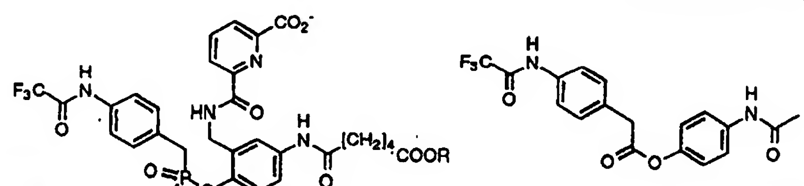
STAGES IN THE PRODUCTION OF CATALYTIC ANTIBODIES

It is appropriate at this stage in the review to consider the stages in production of a catalytic antibody and to put in focus the relative roles of chemistry, immunology, biochemistry, and molecular biology. Nothing less than the full integration of these cognate sciences is essential for the fullest realization of the most difficult objectives in the field of catalytic antibodies. In broad terms, the top section of the flow diagram for abzyme production (Fig. 5) involves chemistry, the right-hand side is immunology, the bottom sector is biochemistry, and molecular biology completes the core of the scheme.

Chemistry

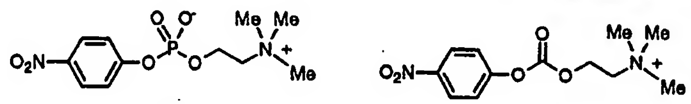
At the outset, chemistry dominates the selection of the process to be investigated (see Scheme 1 later). The chosen reaction should meet most if not all of the following criteria:

[2] It might be helpful to the reader to indicate that the pyridine-2,6-dicarboxylate moiety in [3] was intended for an additional purpose, not used or needed for the activity described in the present scheme (Fig. 4).



[3] [5]

Abzyme identity	Conditions	K_m [5]	k_{cat} [5]	K_i [3]
6D4[x]	pH 8; 25°C	1.9 μM	0.027 s^{-1}	0.16 μM



[4] [6]

Abzyme identity	Conditions	K_m [6]	k_{cat} [6]	K_i [4]
MOPC167	pH 7; 30°C	208 μM	0.007 s^{-1}	5 μM

Fig. 4 Lerner's group used phosphonate [3] as the hapten to raise an antibody which was capable of hydrolysing the ester [5] shown alongside it. Schultz found that naturally occurring antibodies using phosphate [4] as their antigen could hydrolyse the corresponding *p*-nitrophenyl choline carbonate [6]. (Those parts of haptens [3] and [4] required for antibody recognition have been emphasized with bold bonds.)

(a) have a slow but measurable spontaneous rate under ambient conditions;
(b) be well analysed in mechanistic terms;
(c) be as simple as possible in number of reaction steps;
(d) be easy to monitor;
(e) lead to the design of a synthetically accessible TSA of adequate stability.

As we shall see later, most catalytic antibodies achieve rate accelerations in the range 10^3 to 10^6. It follows that for a very slow reaction, e.g. the alkaline hydrolysis of a phosphate diester with $k_{OH} \sim 10^{-11}\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$ direct observation of the reaction is going to be experimentally problematic. Given that concentrations of catalytic antibodies employed are usually in the 1–10 μM range, it has proved far more realistic to target the hydrolysis of an aliphatic ester, with $k_{OH} \sim 0.1\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$ under ambient conditions.

The need for a good understanding of the mechanism of the reaction is well illustrated by the case of amide hydrolysis. Many early enterprises sought to employ transition state analogues (TSAs) that were based on a stable anionic

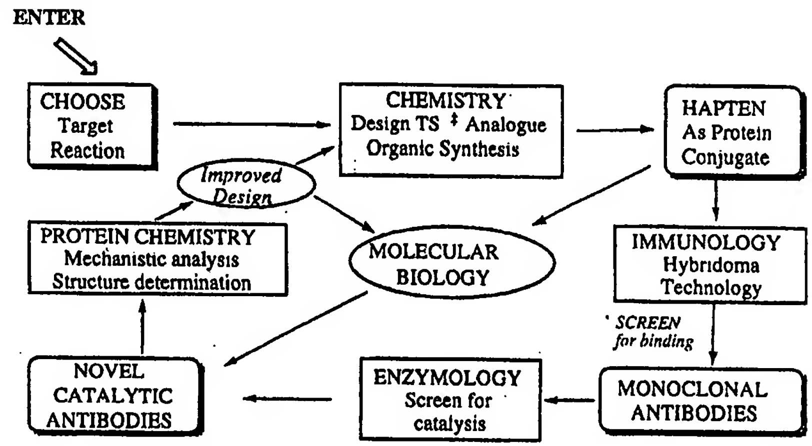


Fig. 5 Stages in the production of a catalytic antibody.

tetrahedral intermediate, as had been successful for ester hydrolysis, and indeed identified catalytic antibodies capable of ester hydrolysis but not of amide cleavage! However, there is good evidence that, for aliphatic amides, breakdown of the tetrahedral intermediate (TI) is the rate-determining step and protonation of the leaving nitrogen is very important, and this must be built into TSA design.

The importance of minimizing the number of covalent steps in the process to be catalysed is rather obvious. Single-step and double-step processes dominate the abzyme scene. However, there is substantial evidence that some acyl transfer reactions involve covalent antibody intermediates and so must proceed by up to four covalent steps. Nonetheless, such antibodies were not elicited by intentional design but rather discovered as a consequence of efficient screening for reactivity (Section 5).

Direct monitoring of the catalysed reaction has most usually been carried out in real time by light absorption or fluorescent emission analysis and some initial progress has been made with light emission detection. The low quantity of abzyme usually available at the screening stage puts a premium on the sensitivity of such methods. However, some work has been carried out of necessity using indirect analysis, e.g. by hplc or nmr.

Finally, this area of research might well have supported a *Journal of Unsuccessful Abzymes*. It is common experience in the field that some three out of four enterprises fail, and for no apparent reason. It is therefore imperative that chemical synthesis of a TSA should not be the rate-determining step of an abzyme project. The average performance target is to achieve hapten synthesis within a year: one or two examples have employed

TSAs that could be found in a chemical catalogue, the most synthetically demanding cases have perforce employed multistep routes of considerable sophistication (e.g. Appendix entry 13.2). And, lastly, the TSA has to survive *in vivo* for at least 2 days to elicit the necessary antigenic response.

Immunology

The interface of chemistry and immunology requires conjugation of multiple copies of the TSA to a carrier protein for production of antibodies by standard monoclonal technology (Köhler and Milstein, 1976). One such conjugate is used for mouse immunization and a second one for ELISA screening purposes. The carrier proteins selected for this purpose are bovine serum albumin (BSA), RMM 67 000, keyhole limpet haemocyanin, RMM 4×10^6, and chicken ovalbumin, RMM 32 000. All of these are basic proteins of high immunogenicity and with multiple surface lysine residues that are widely used as sites for covalent attachment of hapten. Successful antibody production can take some 3 months and should deliver from 20 to 200 monoclonal antibody lines for screening, preferably of IgG isotype.

Screening in early work sought to identify high affinity of the antibody for the TSA, using a process known as ELISA. This search can now be performed more quantitatively by BIAcore analysis, based on surface plasmon resonance methodology (Löfås and Johnsson, 1990). A subsequent development is the catELISA assay (Tawfik *et al.*, 1993), which searches for product formation and hence the identification of abzymes that can generate product.

Methods of this nature are adequate for *screening* sets of hybridomas but not for *selection* from much larger libraries of antibodies. So, most recently, selection methods employing suicide substrates (Section 7) (Janda *et al.*, 1997) or DNA amplification methodology (Fenniri *et al.*, 1995) have been brought into the repertoire of techniques for the direct identification of antibodies that can turn over their substrate. However, the tedious screening of hybridomas remains the mainstay of abzyme identification.

Biochemistry

A family of 100 hybridoma antibodies can typically provide 20 tight binders and these need to be assayed for catalysis. At this stage in the production of an abzyme, the benefit of a sensitive, direct screen for product formation comes into its own. Following identification of a successful catalyst, the antibody is usually recloned to ensure purity and stabilization of the clone, then protein is produced in larger amount (~10 mg) and used for determination of the kinetics and mechanism of the catalysed process by classical biochemistry. Digestion of such protein with trypsin or papain provides fragment antibodies, Fabs, that contain only the attenuated upper limbs of the intact IgG (Fig. 1). It is these components that have been crystallized, in some cases with the substrate analogue, product, or TSA bound in the combining site, and their structures have been determined by X-ray diffraction.

Molecular biology

Only a few abzymes have reached the stage where mutagenesis is being employed to search for improved performance (Miller *et al.*, 1997). Likewise, Hilvert is the first to have reached the stage of redesign of the hapten to attempt the production of antibodies with enhanced performance (Kast *et al.*, 1996). So, the circle of production has now been completed for at least one example, and chemistry can start again with a revised synthetic target.

2 Approaches to hapten design

One can now recognize a variety of strategies in addition to the earliest ones deployed for hapten design. Some of these were presented originally as discrete solutions of the problem of abzyme generation, but it is now recognized that they need not be mutually exclusive either in design or in application. Indeed, more recent work often brings two or more of them together interactively. They can be classified broadly into five categories for the purposes of analysis of their principal design elements. The sequence of presentation of these here is in part related to the chronology of their appearance on the abzyme scene:

1. Transition state analogues
2. Bait and switch
3. Entropy traps
4. Desolvation
5. Functionality augmentation.

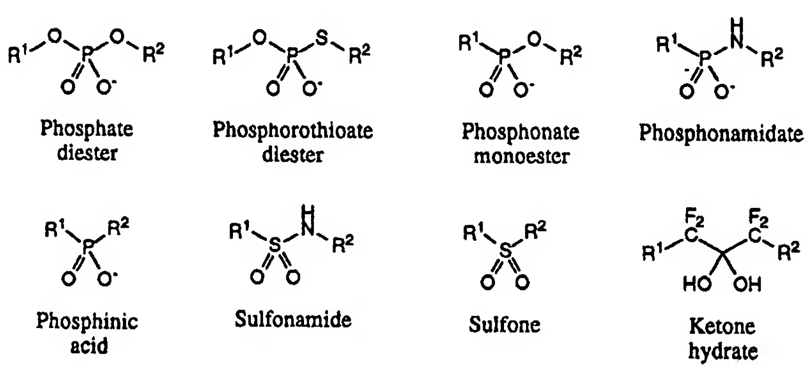
TRANSITION STATE ANALOGUES

As has clearly been shown by the majority of all published work on catalytic antibodies, the original guided methodology, i.e. the design of stable transition state analogues (TSAs) for use as haptens to induce the generation of catalytic antibodies, has served as the bedrock of abzyme research. Most work has been directed at hydrolytic reactions of acyl species, perhaps because of the broad knowledge of the nature of reaction mechanisms for such reactions and the wide experience of deploying phosphoryl species as stable mimics of unstable tetrahedral intermediates. More than 80 examples of hydrolytic antibodies have been reported, including the 47 examples of acyl group transfer to water listed below (Sections 1–5 of the Appendix).

Most such acyl transfer reactions involve stepwise addition of the nucleophile followed by expulsion of the leaving group with a transient, high-energy, tetrahedral intermediate (TI) separating these processes. The faster such reactions generally involve good leaving groups and the addition of the nucleophile is the rate-determining step. This broad conclusion from much detailed kinetic analysis has been endorsed by computation for the hydrolysis of methyl acetate (Teraishi *et al.*, 1994). This places the energy for product formation from an anionic TI^- some 7.6 kcal mol^{-1} lower than for its reversion to reactants. So, for the generation of antibodies for the hydrolysis of aryl esters, alkyl esters, carbonates and activated anilides, the design of hapten has focused on facilitating nucleophilic attack, and with considerable success.

The tetrahedral intermediates used for this purpose initially deployed phosphorus(V) systems, relying on the strong polarization of the P=O bond (arguably more accurately represented as $P^+—O^-$). The range has included many of the possible species containing an ionized P—OH group (Scheme 1). One particularly good feature of such systems is that the $P—O^-$ bond is intermediate in length (1.521 Å) between the $C—O^-$ bond calculated for a TI^- (0.2–0.3 Å shorter) and for the C···O breaking bond in the transition state (some 0.6 Å longer) (Teraishi *et al.*, 1992). Other tetrahedral systems used have included sulfonamides (Shen, 1995) and sulfones (Benedetti *et al.*, 1996), secondary alcohols (Shokat *et al.*, 1990), and α-fluoroketone hydrates (Kitazume *et al.*, 1994).

It is clear that phosphorus-based transition states have had the greatest success, as shown by the many entries in Sections 1–5 of the Appendix. This may be a direct result of their anionic or partial anionic character, a feature not generally available for the other species illustrated in Scheme 1, though α-difluorosulfonamides might reasonably also share this feature as a result of their enhanced acidity.



Scheme 1

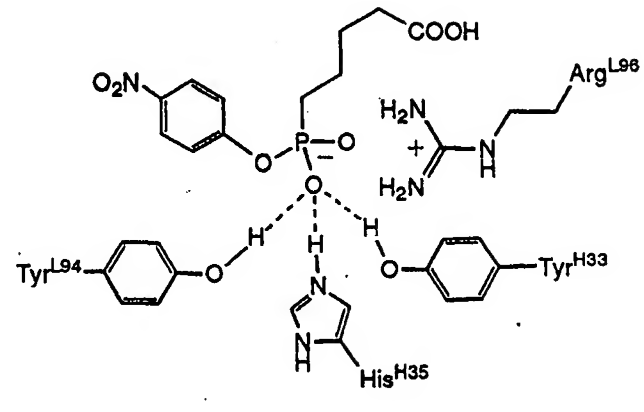


Fig. 6 Binding site details for antibody 48G7 complexed with hapten *p*-nitrophenyl 4-carboxybutanephosphonate (Patten *et al.*, 1996). N.B.: Amino acid residues in antibodies are identified by their presence in the light (L) or heavy (H) chains with a number denoting their sequence position from the N-terminus of the chain.

Not surprisingly, most of the catalytic antibody binding sites examined in structural detail have been found to contain a basic residue that provides a coulombic interaction with these TSAs, for which the prototype is the natural antibody McPC603 to phosphorylcholine, where the phosphate anion is stabilized by coulombic interaction with Arg^{H52} (Padlan *et al.*, 1985). In particular, X-ray structures analysed by Fujii (Fujii *et al.*, 1995) have shown that the protonated His^{H27d} in catalytic antibodies 6D9, 4B5, 8D11 and 9C10 (Appendix entry 1.8) is capable of forming a hydrogen bond to the oxyanion in the transition state for ester hydrolysis.

In similar vein, Knossow has identified His^{H35} located proximate to the oxyanion of *p*-nitrophenyl methylphosphonate in the crystalline binary complex of antibody CNJ206 and TSA, a system designed to hydrolyse *p*-nitrophenyl acetate (cf. Appendix entry 2.7) (Charbonnier *et al.*, 1995). A third example is seen in Schultz's structure of antibody 48G7, which hydrolyses methyl *p*-nitrophenyl carbonate (Appendix entry 3.1c). The hapten *p*-nitrophenyl 4-carboxybutanephosphonate is proximate to Arg^{L96} and also forms hydrogen bonds to His^{H35}, Tyr^{H33} and Tyr^{L94} (Fig. 6) (Patten *et al.*, 1996).

Clearly, the oxyanion hole is now as significant a feature of the binding site of such acyl transfer abzymes as it is already for esterases and peptidases — and not without good reason. Knossow has analysed the structures of three esterase-like catalytic antibodies, each elicited in response to the same phosphonate TSA hapten (Charbonnier *et al.*, 1997). Catalysis for all three is accounted for by transition state stabilization and in each case there is an

oxyanion hole involving a tyrosine residue. This strongly suggests that evolution of immunoglobulins for binding to a single TSA hapten followed by selection from a large hybridoma repertoire by screening for catalysis leads to antibodies with structural convergence. Furthermore, the juxtaposition of X-ray structures of the unliganded esterase mAb D2.3 and its complexes with a substrate analogue and with one of the products provide a complete description of the reaction pathway. D2.3 acts at high pH by attack of hydroxide on the substrate with preferential stabilization of the oxyanion TI^- intermediate, involving one tyrosine and one arginine residue. Water readily diffuses to the reaction centre through a canal that is buried in the protein structure (Gigant *et al.*, 1997). Such a clear picture of catalysis now opens the way for site-directed mutagenesis to improve the performance of this antibody.

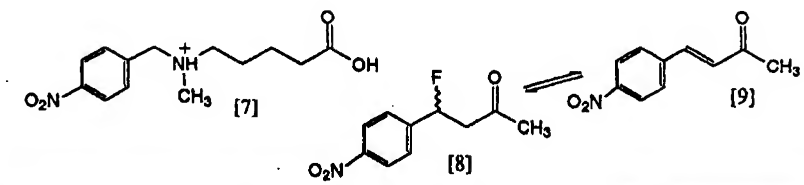
BAIT AND SWITCH

Charge–charge complementarity is an important feature involved in the specific and tight binding of antibodies to their respective antigens. It is the amino acid sequence and conformation of the hypervariable (or complementarity-determining regions, CDRs) in the antibody combining site that determine the interactions between antigen and antibody. This has been exploited in a strategy dubbed "bait and switch" for the induction of antibody catalysts which perform β-elimination reactions (Shokat *et al.*, 1989; Thorn *et al.*, 1995), acyl-transfer processes (Janda *et al.*, 1990b, 1991c; Suga *et al.*, 1994a; Li and Janda, 1995), *cis–trans* alkene isomerizations (Jackson and Schultz, 1991) and dehydration reactions (Uno and Schultz, 1992).

The bait and switch methodology deploys a hapten to act as a "bait". This bait is a modified substrate that incorporates ionic functions intended to represent the coulombic distribution expected in the transition state. It is thereby designed to induce complementary, oppositely charged residues in the combining site of antibodies produced by the response of the immune system to this hapten. The catalytic ability of these antibodies is then sought by a subsequent "switch" to the real substrate and screening for product formation, as described above.

The nature of the combining site of an antibody responding to charged haptens was first elucidated by Grossberg and Pressman (1960), who used a cationic hapten containing a *p*-azophenyltrimethylammonium ion to elicit antibodies with a combining site carboxyl group, essential for substrate binding (as shown by diazoacetamide treatment).

The first example of "bait and switch" for catalytic antibodies was provided by Shokat (Shokat *et al.*, 1989), whose antibody 43D4-3D12 raised to hapten [7] was able to catalyse the β-elimination of [8] to give the *trans*-enone [9] with a rate acceleration of 8.8×10^4 over background (Fig. 7; Appendix entry 8.1).



Abzyme identity	Conditions	K_m [8]	k_{cat} [8]	K_i [7]
43D4-3D12	pH 6; 37°C	182 μM	$0.003\ s^{-1}$	0.29 μM

Fig. 7 Using the "bait and switch" principle, hapten [7] elicited an antibody, 43D4-3D12, which catalysed the β-elimination of [8] to a *trans*-ene-one [9]. The carboxyl function in [7] is necessary for its attachment to the carrier protein.

Subsequent analysis has identified a carboxylate residue, Glu^{46H} as the catalytic function induced by the cationic charge in [7] (Shokat *et al.*, 1994).

A similar "bait and switch" approach has been exploited for acyl-transfer reactions (Janda *et al.*, 1990b, 1991c). The design of hapten [10] incorporates both a transition state mimic and the cationic pyridinium moiety, designed to induce the presence of a potential general acid/base or nucleophilic amino acid residue in the combining site, able to assist in catalysis of the hydrolysis of substrate [11] (Appendix entry 2.6).

Some 30% of all of the monoclonal antibodies obtained using hapten [10] were catalytic, and so the work was expanded to survey three other antigens based on the original TSA design (Janda *et al.*, 1991c). The carboxylate anion in [12] was designed to induce a cationic combining site residue, whilst the quaternary ammonium species [13] combines tetrahedral mimicry and positive charge in the same locus. Finally, the hydroxyl group in [14] was designed to explore the effects of a neutral antigen (Fig. 8).

Three important conclusions arose from this work.

(i) A charged functionality is crucial for catalysis.
(ii) Catalytic antibodies are produced from targeting different regions of the binding site with positive and negative haptens (though more were obtained in the case of the cationic hapten used originally).
(iii) The combination of charge plus mimicry of the transition state is required to induce hydrolytic esterases.

Esterolytic antibodies have also been produced by Suga using a different "bait and switch" strategy (Appendix entry 2.1) (Suga *et al.*, 1994a). A 1,2-aminoalcohol function was designed for generating not only esterases but also amidases. Of three haptens synthesized, [15], [16] and [17], two contained

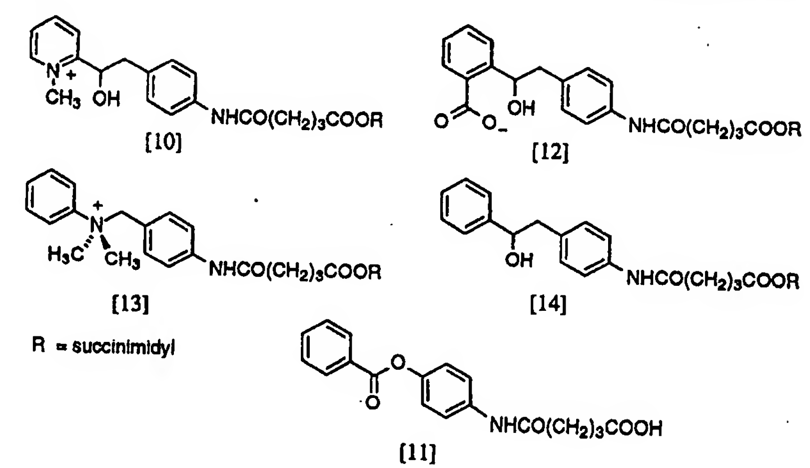


Fig. 8 The original hapten [10] demonstrated the utility of the "bait and switch" strategy in the generation of antibodies to hydrolyse the ester substrate [11]. Three haptens, [12]–[14], were designed to examine further the effectiveness of point charges in amino acid induction. Both charged haptens, [12] and [13], produced antibodies that catalysed the hydrolysis of [11], whereas the neutral hapten, [14], generated antibodies which bound the substrate unproductively.

ammonium cations and one a protonated amine, in order to elicit an anionic combining site for covalent catalysis. The outcome was interpreted as suggesting that haptens containing an NMe_3^+ group were too demanding sterically, so that the induced anionic amino acid residues in the antibody binding pocket were too distant to provide nucleophilic attack at the carbonyl carbon of substrate [18]. An alternative explanation may be that coulombic interactions lacking any hydrogen-bonding capability will not be sufficiently short range for the purpose intended.

The use of secondary hydroxyl groups in the haptens [15] and [16] was designed to mimic the tetrahedral geometry of the transition state (as in Janda's work), while the third hapten [17] replaced the neutral OH with an anionic phosphate group, designed to elicit a cationic combining site residue to stabilize the transition state oxyanion. However, this function in [17] may have proved too large to induce a catalytic residue close enough to the developing oxyanion, since weaker catalysis was observed relative to haptens [15] and [16] ($k_{cat}/k_{uncat} = 2.4 \times 10^3$, 3.3×10^3, and $\sim 1 \times 10^3$ for [15], [16], and [17] respectively) (Fig. 9).

To achieve catalysis employing both acid and basic functions, an alternative zwitterionic hapten was proposed in which the anionic phosphoryl core is incorporated alongside the cationic ammonium moiety (cf. [17]) (Suga *et al.*,

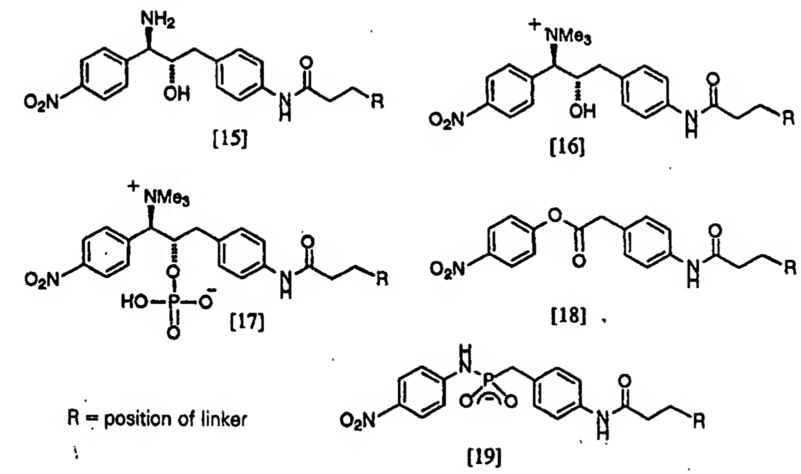
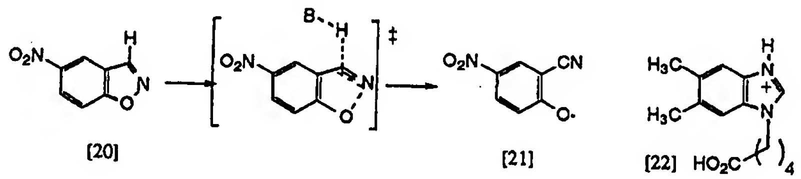


Fig. 9 Three haptens, [15]–[17], containing a 1,2-aminoalcohol functionality were investigated as alternatives for esterase and amidase induction. Of antibodies raised against hapten [15], 50% were shown to catalyse the hydrolysis of ester [18], thereby establishing the necessity for a compact haptenic structure. Hapten [19] along with [16] was employed in a heterologous immunization programme to elicit both a general and acid/base function in the antibody binding site.

1994b). The difficulty in synthesizing such a target hapten can be overcome by stimulating the immune system first with the cationic and then with the anionic point charges using the two structurally related haptens [16] and [19], respectively. Such a sequential strategy has been dubbed "heterologous immunization" (Fig. 9) and the results of this strategy were compared with those from the individual use of haptens [16] and [19] in a "homologous immunization" routine. Of 48 clones produced as a result of the homologous protocols, 7 were found to be catalytic, giving rate enhancements up to 3×10^3. By contrast, 19 of the 50 clones obtained using the heterologous strategy displayed catalysis, the best being up to 2 orders of magnitude better.

A final example of the bait and switch strategy (Thorn *et al.*, 1995) focuses on the base-promoted decomposition of substituted benzisoxazole [20] to give cyanophenol [21] (Appendix entry 8.4). A cationic hapten [22] was used to mimic the transition state geometry of all reacting bonds. It was anticipated that if the benzimidazole hapten [22] induced the presence of a carboxylate in the binding site, it would be ideally positioned to make a hydrogen bond to the N-3 proton of the substrate. The resultant abzymes would thus have general base capability for abstracting the H-3 in the substrate (Fig. 10).

Two monoclonals, 34E4 and 35F10, were found to catalyse the reaction with a rate acceleration greater than 10^8, while the presence of a carboxylate-



[20] [21] [22]

Fig. 10 The use of a cationic hapten [22] mimics the transition state of the base-promoted decomposition of substituted benzisoxazole [20] to cyanophenol [21] and also acts as a "bait" to induce the presence of an anion in the combining site that may act as a general base.

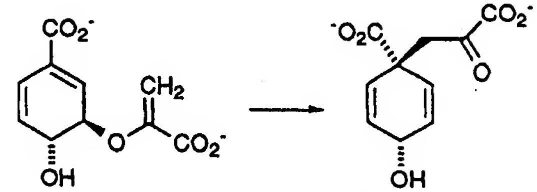
containing binding site residue was confirmed by pH–rate profiles and covalent modification by a carbodiimide, which reduced catalysis by 84%.

The bait and switch tactic clearly illustrates that antibodies are capable of a coulombic response that is potentially orthogonal to the use of transition state analogues in engendering catalysis. By variations in the hapten employed, it is possible to fashion antibody combining sites that contain individual residues to deliver intricate mechanisms of catalysis.

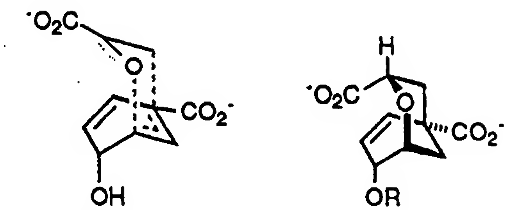
ENTROPY TRAPS

Rotational entropy

An important component of enzyme catalysis is the control of translational and rotational entropy in the transition state (Page and Jencks, 1971). This is well exemplified for unimolecular processes by the enzyme chorismate mutase, which catalyses the isomerization of chorismic acid [23] into prephenic acid [24]. This reaction proceeds through a cyclic transition state having a pseudo-diaxial conformation [25] (Addadi *et al.*, 1983). With this analysis, Bartlett designed and synthesized a transition state analogue [26] which proved to be a powerful inhibitor for the enzyme (Bartlett and Johnson, 1985). X-ray structures of mutases from *Escherichia coli* (Lee *et al.*, 1995), *Bacillus subtilis* (Chook *et al.*, 1993, 1994) and *Saccharomyces cerevisiae* (Xue and Lipscomb, 1995) complexed to [26] show completely different protein architectures although the bacterial enzymes have similar values of k_{cat}/k_{uncat} (3×10^6) and of K_I for [26]. It appears that these enzymes exert their catalysis through a combination of conformational control and enthalpic lowering. Supporting this, Hillier has carried out a hybrid quantum-mechanical/molecular mechanics calculation on the *B. subtilis* complex with substrate [23]. He concluded that interactions between protein and substrate are maximal close to the transition state [25] and lead to a lowering of the energy barrier greater than is needed to produce the observed rate acceleration (Davidson and Hillier, 1994).



Chorismate [23] Prephenate [24]



Transition state [25] TS Analogue [26]

Schultz employed TSA [26] as a hapten to generate antibodies to catalyse this same isomerization reaction [23]–[24] (Jackson *et al.*, 1988). His kinetic analysis of one purified antibody revealed that it increases the entropy of activation of the reaction by 12 cal mol^{-1} K^{-1} (Table 1, Antibody 11F1-2E11, Appendix entry 13.2b), and gives a rate enhancement of 10^4. He suggested that this TSA induces a complementary combining site in the abzyme that constrains the reactants into the correct conformation for the [3,3]-sigmatropic reaction and designated this strategy as an "entropic trap".

Table 1 Kinetic and thermodynamic parameters for the spontaneous, enzyme-catalysed and antibody-catalysed conversion of chorismic acid [23] into prephenic acid [24].

Catalyst	Relative rate	$\Delta G^{\ddagger}$/ kcal mol^{-1}	$\Delta H^{\ddagger}$/ kcal mol^{-1}	$\Delta S^{\ddagger}$/ cal mol^{-1} K^{-1}	K_m [23]	k_{cat} [23]	K_I [26]
Spontaneous[a]	1	24.2	20.5	−12.9			
Chorismate Mutase[b]	3×10^6	15.9	15.9	0	45 μM	1.35 s^{-1}	75 μM
Antibody 1F7[c]	250	21.3	15.0	−22	51 μM	0.072 min^{-1}	600 nM
11F1-2E11[d]	10000	18.7	18.3	−1.2	260 μM	0.27 min^{-1}	9.0 μM

[a]At 25°C. [b]*E. coli* enzyme at 25°C. [c]pH 7.5; 14°C. [d]pH 7.0; 10°C.

Hilvert's group used the same hapten [26] with a different spacer to generate an antibody catalyst which has very different thermodynamic parameters. It has a high entropy of activation but an enthalpy lower than that of the wild-type enzyme (Table 1, Antibody 1F7, Appendix entry 13.2a) (Hilvert *et al.*, 1988; Hilvert and Nared, 1988). Wilson has determined an X-ray crystal structure for the Fab′ fragment of this antibody in a binary complex with its TSA (Haynes *et al.*, 1994) which shows that amino acid residues in the active site of the antibody catalyst faithfully complement the components of the conformationally ordered transition state analogue (Fig. 11) while a trapped water molecule is probably responsible for the adverse entropy of activation. Thus it appears that antibodies have emulated enzymes in finding contrasting solutions to the same catalytic problem.

Further examples of catalytic antibodies that are presumed to control rotational entropy are AZ-28, which catalyses an oxy-Cope [3.3]-sigmatropic rearrangement (Appendix entry 13.1) (Braisted and Schultz, 1994; Ulrich *et al.*, 1996) and 2E4, which catalyses a peptide bond isomerization (Appendix entry 13.3) (Gibbs *et al.*, 1992b; Liotta *et al.*, 1995). Perhaps the area for the greatest opportunity for abzymes to achieve control of rotational entropy is in the area of cationic cyclization reactions (Li *et al.*, 1997). The achievements of the Lerner group in this area (Appendix entries 15.1–15.4) will be discussed later in this article (Section 6).

Translational entropy

The classic example of a reaction that demands control of translational entropy is surely the Diels–Alder cycloaddition. It is accelerated by high pressure and by solutions 8 M in LiCl (Blokzijl and Engberts, 1994; Ciobanu and Matsumoto, 1997; Dell, 1997) and proceeds through an entropically disfavoured, highly ordered transition state, showing large activation entropies in the range of −30 to −40 cal mol^{-1} K^{-1} (Sauer, 1966).

While it is one of the most important and versatile transformations available to organic chemists, there is no unequivocal example of a biological counterpart. Hence, attempts to generate antibodies which could catalyse this reaction were seen as an important target. The major task in producing a "Diels–Alderase" antibody lies in the choice of a suitable haptenic structure, because the transition state for the reaction resembles product more closely than reactants (Fig. 12). The reaction product itself is an inappropriate hapten because it is likely to result in severe product inhibition of the catalyst, thereby preventing turnover.

Tetrachlorothiophene dioxide (TCTD) [27] reacts with *N*-ethylmaleimide [28] to give an unstable, tricyclic intermediate [29] that spontaneously extrudes SO_2 to give a dihydrophthalimide as the bicyclic adduct [30] (Raasch, 1980). This led to the design of hapten as a bridged dichloro-tricycloazadecene derivative [31] which closely mimics the high-energy intermediate [29] whilst

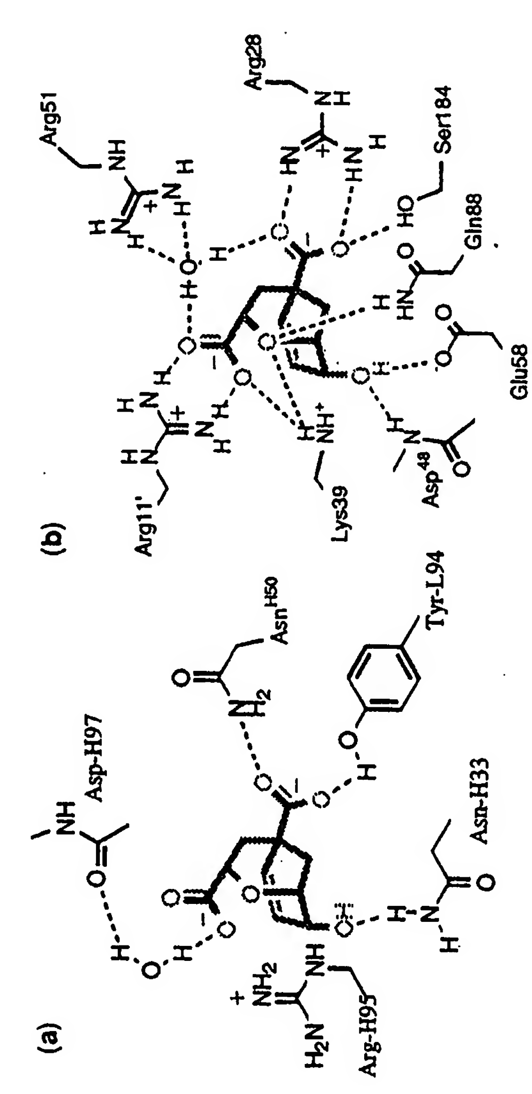
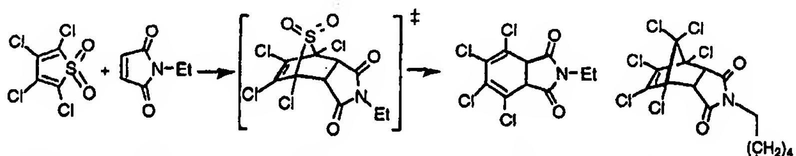


Fig. 11 Schematic diagrams of X-ray crystal structures show the hydrogen-bonding (dashed lines) and electrostatic interactions between the transition state analogue [26] (in grey) with relevant side chains of (a) antibody 1F7 (Haynes *et al.*, 1994) and (b) the active site of the *E. coli* enzyme (Lee *et al.*, 1995).



[27] [28] [29] [30] [31]

Fig. 12 The Diels–Alder cycloaddition of TCTD [27] and [28] proceeds through an unstable intermediate [29] which spontaneously extrudes SO_2 to give the dihydrophthalimide adduct [30]. Hapten [31] was designed as a stable mimic of [29] that would be sufficiently different from product [30] to avoid product inhibition of the antibody catalyst.

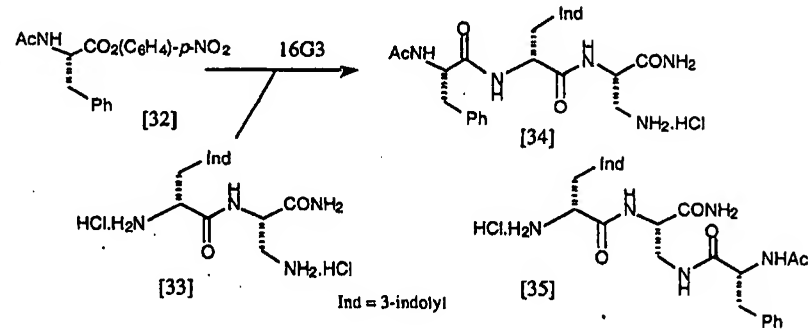
being sufficiently different from the product [30] to avoid the possibility of end-product inhibition (Hilvert *et al.*, 1989).

Several antibodies raised to the hapten [31] accelerated the Diels–Alder cycloaddition between [27] and [28]. The most efficient of these, 1E9, performs multiple turnovers, showing that product inhibition has been largely avoided. Comparison of k_{cat} with the second-order rate constant for the uncatalysed reaction ($k_{uncat} = 0.04\ \text{M}^{-1}\ \text{min}^{-1}$, 25°C) gives an effective molarity,[3] EM, of 110 M (Appendix entry 17.1) (Hilvert *et al.*, 1989). This value is several orders of magnitude larger than any attainable concentration of substrates in aqueous solution, and therefore the antibody binding site confers a significant entropic advantage over the bimolecular Diels–Alder reaction.

A number of further examples of Diels–Alder catalytic antibodies have been described (Appendix entries 17.2–17.5) and they must needs benefit from the same entropic advantage over spontaneous reactions, albeit without Hilvert's ingenious approach to avoiding product inhibition. Their success in achieving control of regio- and stereo-chemistry will be discussed later (Section 6).

Of greater long-term significance is the control of translational entropy for antibody-catalysed synthetic purposes. Benkovic's description of an antibody ligase capable of joining an activated amino acid (e.g. [32]) to a second amino acid to give a dipeptide and to a dipeptide (e.g. [33]) to give a tripeptide with only low product inhibition is particularly significant (Scheme 2) (Appendix entry 18.4) (Smithrud *et al.*, 1997). Antibody 16G3 can achieve 92% conversion of substrates for tripeptide formation and 70% for tetrapeptide synthesis within an assay time of 20 min. A concentration of 20 μM antibody can produce a 1.8 mM solution of a dipeptide in 2 h. The very good regio-control of the catalysed process is shown by the 80:1 ratio of formation

[3] The EM is equivalent to the concentration of substrate that would be needed in the uncatalysed reaction to achieve the same rate as achieved by the antibody ternary complex (Kirby, 1980).



[32] [33] [34] [35] Ind = 3-indolyl

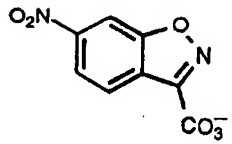
Scheme 2

of the programmed peptide [34] compared to the unprogrammed product [35], whereas the uncatalysed reaction gives a 1:1 ratio.

DESOLVATION

The Kemp decarboxylation of 6-nitro-3-carboxybenzisoxazole [36] is a classic example of rate acceleration by desolvation. Moving from water to a less polar environment can effect a 10^7-fold rate acceleration, which has been ascribed to a combination of (i) substrate destabilization by loss of hydrogen-bonding to solvent and (ii) transition state stabilization in a dipolar aprotic solvent (Kemp *et al.*, 1975). Both Hilvert and Kirby have sought to generate abzymes for this process (Appendix entry 9.1) (Lewis *et al.*, 1991; Sergeeva *et al.*, 1996). Hilvert generated several antibodies using TSA [37] and the best, 25E10, gave a rate acceleration of 23 200 for decarboxylation of [36], comparable to rate accelerations found in other mixed solvent systems but much less than for hexamethylphosphoric triamide ($\times 10^8$). In particular, it is of some concern that the K_m for this antibody is as high as 25 mM, which reflects the tenuous relationship between the hapten design and the substrate/transition state structure. Unfortunately, apparently better-designed TSAs, e.g. [38] (Sergeeva *et al.*, 1996), fared worse in outcome, probably through the absence of a counter cation in the binding site. This may offer an opportunity for protein engineering to induce the presence of an *N,N,N*-trimethyllysine residue in the active site to provide a non-hydrogen-bonding salt pair.

Selenoxide *syn*-eliminations are another reaction type favoured by less polar solvents (Reich, 1979). The planar 5-membered, pericyclic transition state for *syn*-elimination of [39] was mimicked by the racemic proline-based *cis*-hapten [39] to give 28 monoclonal antibodies (Appendix entry 8.5) (Zhou *et al.*, 1997). Abzyme SZ-*cis*-42F7 converted substrate [40] exclusively into



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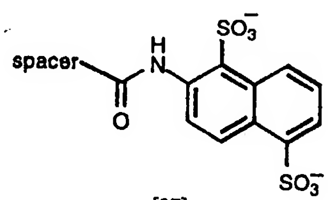

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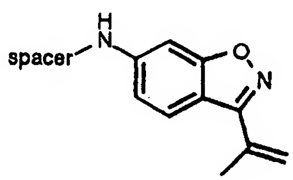

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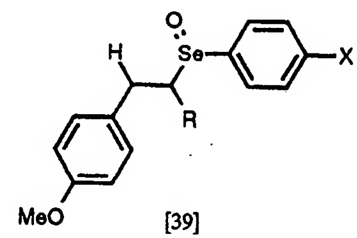

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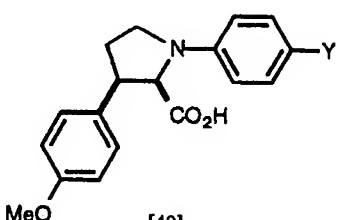
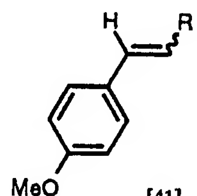


[40]



[41]

trans-anethole [41] with an enhancement ratio (ER) of 62 ($R = Me$, $X = NO_2$) and with a low K_m of 33 μM. Abzyme SZ-*cis*-39C11 gave a good acceleration, k_{cat} 0.036 min^{-1}, k_{cat}/K_m 2400 M^{-1} min^{-1} (substrate [40], $R = H = X$) comparable to the rate in 1,2-dichloroethane solution. Unexpectedly, the catalytic benefit appears to be mainly enthalpic both for the antibody and for the solvent switch, as shown by the data in Table 2.

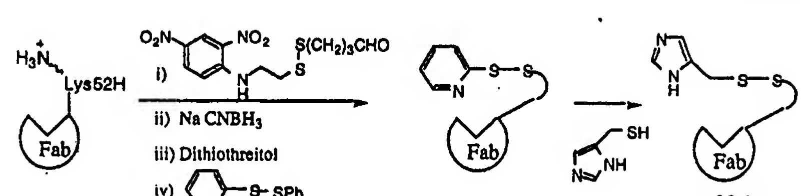
AUGMENTATION OF CHEMICAL FUNCTIONALITY

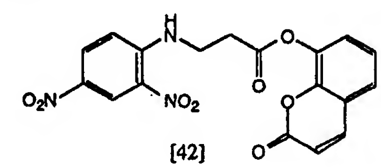
Several antibodies have been modified to incorporate natural or synthetic groups to aid catalysis (Pollack *et al.*, 1988). Pollack and Schultz reported the first example of a semi-synthetic abzyme through the introduction of an

Table 2 Parameters at 25°C for the *syn*-elimination of selenoxide [39] ($R = X = H$) in water, DCM, and catalysed by antibody SZ-*cis*-39C11.

Catalyst	$\Delta G^{\ddagger}$	$\Delta H^{\ddagger}$/ kcal mol^{-1}	$\Delta S^{\ddagger}$/ cal mol^{-1} K^{-1}	k_{cat}/K_m M^{-1} min^{-1}	k_{cat} (k_{obs})[a]/ min^{-1}	ER
Water	26.3 ± 0.15	26.3 ± 0.15	+0.014 ± 0.47		1.6×10^{-5}	
SZ-*cis*-39C11	22.2 ± 1.2	19.7 ± 1.2	−7.8 ± 4.1	2400	3.5×10^{-2}	2200
DCM	21.8 ± 0.5	20.3 ± 0.5	−4.8 ± 1.7		4.4×10^{-2}	2750

[a]25°C.





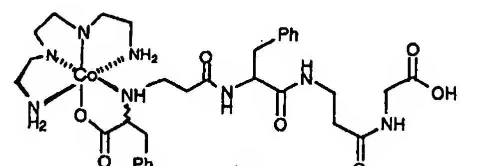
[42]

Fig. 13 A semi-synthetic abzyme. Selective derivatization of lysine-52 in the heavy chain of MOPC315 creates a thiol, then bonded to an imidazole, which gives an abzyme capable of improved hydrolysis of coumarin ester [42] with $k_{cat} = 0.052$ min^{-1}.

imidazole residue into the catalytic site by selective modification of the thiol-containing antibody MOPC315 (Pollack and Schultz, 1989). This yielded a chemical mutant capable of hydrolysing coumarin ester [42] with k_{cat} 0.052 min^{-1} at pH 7.0, 24°C. Incorporation of the nucleophilic group alone was previously shown to accelerate hydrolysis of the ester by a factor of 10^4 over background controls (Pollack *et al.*, 1988).

The process of modification is shown in Fig. 13. Lys-52 is first derivatized with 4-thiobutanal and then a catalytic imidazole is bonded through a disulfide bridge into the active site. This can now act as a general base/nucleophile in the hydrolysis of [42], as was verified first by the pH–rate profile and then by complete deactivation of the antibody by diethyl pyrocarbonate (an imidazole-specific inactivating reagent).

The first success in sequence-specific peptide cleavage by an antibody was claimed by Iverson (Iverson and Lerner, 1989). He used hapten [43] containing an inert Co^{III}(trien) complexed to the secondary amino acid of a



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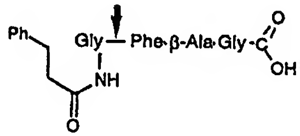


[44]

Fig. 14 A metal complex [43] used as hapten to raise antibodies capable of incorporating metal co-factors to facilitate the cleavage of [44] at the position indicated (↓).

tetrapeptide. This approach was planned in the expectation of eliciting monoclonal antibodies with a binding site that could simultaneously accommodate a substrate molecule and a kinetically labile complex such as Zn^{II}(trien) or Fe^{III}(trien), designed to provide catalysis. Much early work by Buckingham and Sargeson had shown that such cobalt complexes are catalytic for amide hydrolysis via polarization of the carbonyl group, through nucleophilic attack of metal-bound hydroxide, or by a combination of both processes (Sutton and Buckingham, 1987; Hendry and Sargeson, 1990).

Of 13 peptidolytic monoclonals, 287F11 was selected for further analysis. At pH 6.5, cleavage of substrate [44] was observed with a variety of metal complexes. The Zn^{II}(trien) complex was the most efficient, with 400 turnovers per antibody combining site and a turnover number of $6 \times 10^{-4}\,s^{-1}$ (Fig. 14). While this approach is undoubtedly ingenious, there are some doubts about its actual performance. The site of cleavage of peptide [44] is not between the N-terminal phenylalanine and glycine, as expected from the design of the hapten, but rather between glycine and the internal phenylalanine. Moreover, attempts to repeat this work have not been overly successful.

A major achievement in augmenting the chemical potential of antibodies has been in the area of redox processes. Many examples now exist of stereoselective reductions, particularly recruiting sodium cyanoborohydride (Appendix Section 22). A growing number of oxidation reactions can now be catalysed by abzymes, with augmentation from oxidants such as hydrogen peroxide and sodium periodate (Appendix Section 21).

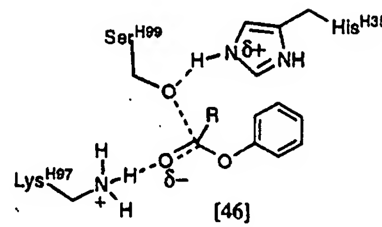
3 Spontaneous features of antibody catalysis

While the presentation thus far has emphasized the programmed relationship of hapten design and consequent antibody catalytic activity, there is a growing number of examples where the detailed examination of catalysis reveals mechanistic features that were not evidently design features of the system at the outset. Such discoveries are clearly a strength rather than a weakness of

the abzyme field, and two of these outturns are described in the following sections.

SPONTANEOUS COVALENT CATALYSIS

The nucleophilic activity of serine in the hydrolysis of esters and amides by many enzymes is one of the classic features of covalent catalysis by enzymes. So it was perhaps inevitable that an antibody capable of catalysing the hydrolysis of a phenyl ester should emerge having the same property. Scanlan has provided just that example with evidence from kinetic and X-ray structural analysis to establish that the hydrolysis of phenyl (*R*)-*N*-formylnorleucine [45] proceeds via an acyl antibody intermediate with abzyme 17E8 (Appendix entry 2.3) (Zhou *et al.*, 1994). The antibody reaction has a bell-shaped pH–rate profile corresponding to ionizable groups of pK_a 9.1 and 10.0. On the basis of X-ray analysis, the latter appears to be Lys^{H97}, while a candidate for the former is Tyr^{H101}. This system is deemed to activate Ser^{H99} as part of a catalytic diad with His^{H35} (Scheme 3 [46]). In addition to the kinetic and structural evidence



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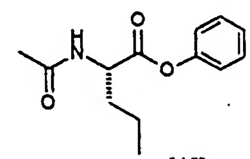


[45]

Scheme 3

for this claim, a trapping experiment with hydroxylamine generated a mixture of amino acid and amino hydroxamic acid products from substrate [45] in the presence of antibody.

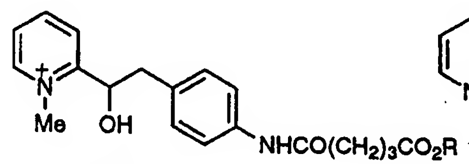
In a similar vein, antibody NPN43C9 appears to employ a catalytic histidine, His^{L91}, as a nucleophilic catalyst in the hydrolysis of a *p*-nitrophenyl phenylacetate ester, as discussed in detail below (Section 5; Appendix entry 2.8) (Gibbs *et al.*, 1992a; Chen *et al.*, 1993).

SPONTANEOUS METAL ION CATALYSIS

Janda and Lerner sought to establish that a metal ion or coordination complex need not be included within the hapten used for the induction of abzymes so that they can (i) bind a metallo-complex and thereby (ii) provide a suitable

environment for catalysis (Wade *et al.*, 1993). The pyridine ester [48] was screened as a substrate for 23 antibodies raised against [47] as hapten. Antibody 84A3 proved to be capable of hydrolysis of [48] only in the presence of zinc, with a rate enhancement of 12 860 over the spontaneous rate and 1230 over that seen in the presence only of zinc. Other metals, Cd^{2+}, Co^{2+}, Ni^{2+}, were without activity. The affinity of 84A3 for the substrate was high at 3.5 μM, whereas the affinity for zinc in the presence of substrate was only 840 μM. This is far weaker than any affinity of real use for the incorporation of metal ion activity into the catalytic antibody repertoire (plasma [Zn^{2+}] is 17.2 μM). However, the resources of mutagenesis can readily be targeted on this problem with expectations of success.

Given the great importance of the metalloproteinases, it seems inevitable that further work will be directed at this key area either by designed or opportunistic incorporation of metal ions into the catalytic apparatus of abzymes.



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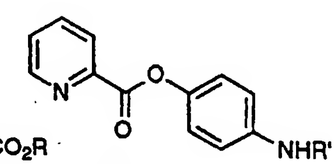


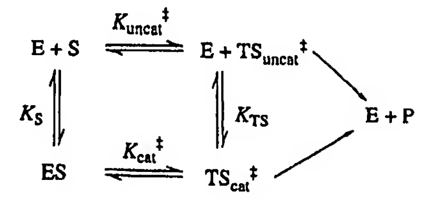
[48]

4 Performance analysis of catalytic antibodies

In the first years of abzyme research, a majority of examples was concerned with acyl group transfer reactions. Many of these endeavours have been based on mimicry of the high-energy, tetrahedral intermediate that lies along such reaction pathways (Section 2) and which, though not truly a "transition state analogue", provides a realistic target for production of a stable TSA. Most, though not all, were themselves based on four-coordinate phosphoryl centres.

In 1991, Jacobs analysed 18 examples of antibody catalysis of acyl-transfer reactions as a test of the Pauling concept, i.e. delivering catalysis by TS‡ stabilization. The range of examples included the hydrolysis of aryl carbonates and of both aryl and alkyl esters. In some cases more than one reaction was catalysed by the same antibody, in others the same reaction was catalysed by different antibodies.

Much earlier, Wolfenden (Westerick and Wolfenden, 1972) and Thompson (1973), established a criterion for enzyme inhibitors working as TSAs. They proposed that such activity should be reflected by a linear relationship between the inhibition constant for the enzyme K_i and its inverse second-



From TS‡ Theory $\dfrac{k_{cat}}{k_{uncat}} = \dfrac{K_{cat}^\ddagger}{K_{uncat}^\ddagger}$ From cycle $\dfrac{K_{cat}^\ddagger}{K_{uncat}^\ddagger} = \dfrac{K_S}{K_{TS}}$ *If* $K_S \approx K_m$ *and if* $K_{TS} \approx K_i$ Then $\dfrac{k_{cat}}{k_{uncat}} = \dfrac{K_S}{K_{TS}}$

$$\boxed{\text{Thus} \quad \frac{k_{cat}}{k_{uncat}} = \frac{K_m}{K_i}}$$

Fig. 15 A thermodynamic cycle linked to transition state theory gives an equation relating the enhancement ratio for a biocatalysed process to the ratio of equilibrium constants for the complex between the biocatalyst and (i) substrate and (ii) the transition state for the reaction. These two values can be estimated as K_m and K_i for the TSA, respectively.

order rate constant, K_m/k_{cat}, for pairs of inhibitors and substrates that differ in structure only at the TSA/substrate locus. That has been well validated, *inter alia*, for phosphonate inhibitors of thermolysin (Bartlett and Marlowe, 1983) and pepsin (Bartlett and Giangiordano, 1996). In order to apply such a criterion to a range of catalytic antibodies, Jacobs assumed firstly that the spontaneous hydrolysis reaction proceeds via the same TS‡ as that for the antibody-mediated reaction and secondly that all corrective factors due to medium effects are constant. By treating the hydrolysis reactions as pseudo-first-order processes, one can derive a simple relationship with approximations of K_{TS} and K_S to provide a mathematical statement in terms of K_i, K_m, k_{cat} and k_{uncat} (Fig. 15) (Wolfenden, 1969; Jencks, 1975; Benkovic *et al.*, 1988; Jacobs, 1991).

A log–log plot using K_i, K_m, k_{cat} and k_{uncat} data from the 18 separate cases of antibody catalysis exhibited a linear, albeit scattered, correlation over four orders of magnitude and with a gradient of 0.86 (Fig. 16).[4] Considering the assumptions made, this value is sufficiently close to unity to suggest that the antibodies do stabilize the transition state for their respective reactions. However, even the highest k_{cat}/k_{uncat} value of 10^6 in this series (Tramontano *et al.*, 1988) barely compares with enhancement ratios seen for weaker enzyme catalysts (Lienhard, 1973).

[4]It may also be worth mentioning here that many early estimates of K_d for the affinity of the antibody to their TSA were upper limits, being based on inhibition kinetics using concentrations of antibody that were significantly higher than the true K_i being determined.

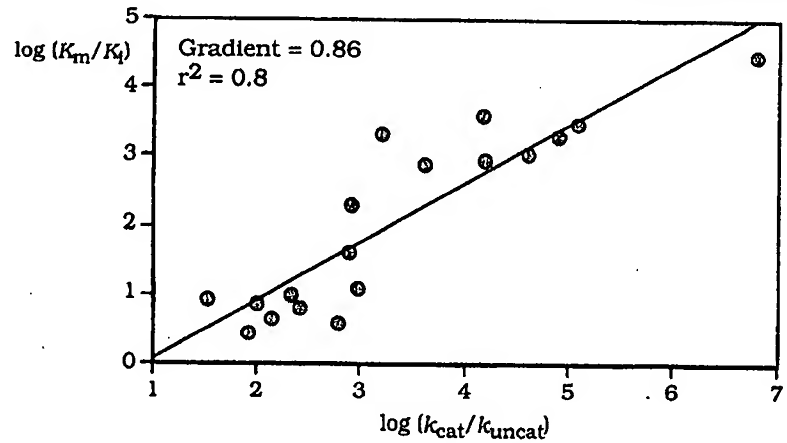


Fig. 16 Jacobs' correlation between the enhancement ratio (k_{cat}/k_{uncat}) and the relative affinity for the TSA with respect to the substrate (K_m/K_i) (Jacobs, 1991). The slope is an unweighted linear regression analysis.

The fact that many values of K_m/K_i fall below the curve (Fig. 16) suggested that interactions between the antibody and the substrate are largely passive in terms of potential catalytic benefit. This conclusion exposes a serious limitation in the design of haptens, were that to be restricted solely to the transition state concept. It is well known that enzymes utilize a range of devices to achieve catalysis as well as dynamic interactions to guide substrate towards the transition state, which is then selectively stabilized. However, as has been illustrated above, the original concept of transition state stabilization has been augmented by a range of further approaches in the generation of catalytic antibodies and with considerable success.

A second use of this type of analysis has been presented by Stewart and Benkovic (1995). They showed that the observed rate accelerations for some 60 antibody-catalysed processes can be predicted from the ratio of equilibrium binding constants to the catalytic antibodies for the reaction substrate, K_m, and for the TSA used to raise the antibody, K_i. In particular, this approach supports a rationalization of product selectivity shown by many antibody catalysts for disfavoured reactions (Section 6) and predictions of the extent of rate accelerations that may be ultimately achieved by abzymes. They also used the analysis to highlight some differences between mechanism of catalysis by enzymes and abzymes (Stewart and Benkovic, 1995). It is interesting to note that the data plotted (Fig. 17) show a high degree of scatter with a correlation coefficient for the linear fit of only 0.6 and with a slope of 0.46, very different from the "theoretical slope" of unity. Perhaps of greatest significance are the

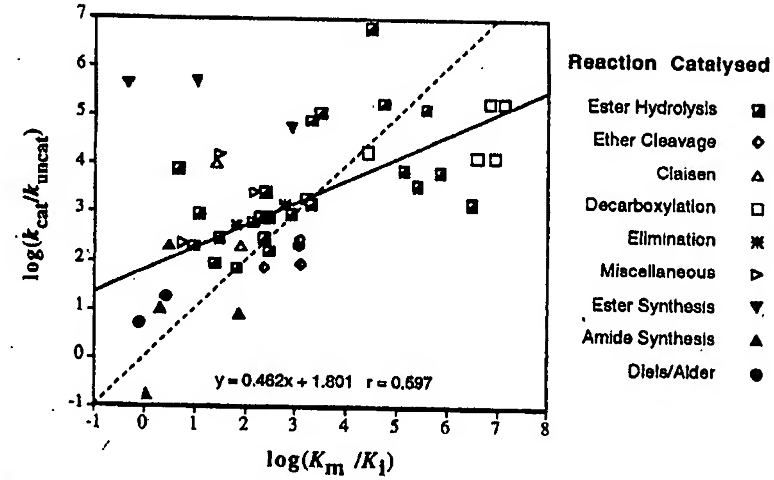


Fig. 17 The Stewart–Benkovic plot of rate enhancement vs relative binding of substrate and TSA for 60 abzyme-catalysed reactions (Stewart and Benkovic, 1995). The theoretical unit slope (---) diverges from the linear regression slope (——) for these data (for which the equation is shown).

many positive deviations from the general pattern. These appear to show that antibody catalysis can achieve rather more than is predicted from catalysis through transition state stabilization alone.

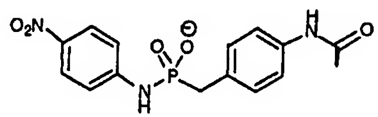
5 A case study: NPN43C9 – an antibody anilidase

At this point, we can integrate much of what has been discussed above in a single case study. Antibody NPN43C9 was reported in 1988 as the first example of catalysis of hydrolysis of an amide bond, in fact of an active anilide. Its structure and mode of action have been well studied (Janda *et al.*, 1988b), which makes it an appropriate example for this purpose.

ANTIBODY PRODUCTION

Hapten design

Amide hydrolysis at alkaline pH involves a tetrahedral anionic intermediate, which was mimicked by the transition state analogue [49], an *N*-aryl arylphosphonamidate, appropriately related to substrate anilide [50] (Fig. 18) (Appendix entry 2.8).



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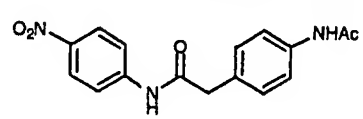


[50]

Abzyme identity	Conditions	K_m [50]	k_{cat} [50]	K_d [49]
NPN43C9	pH 9; 37°C	370 μM	0.05 min^{-1}	0.8 nM

Fig. 18 Antibody NPN43C9, raised against the phosphonamidate hapten [49], was capable of accelerating the hydrolysis of the anilide [50].

Whilst hapten [49] satisfies the stereoelectronic requirements for the TI$^-$ for amide hydrolysis, the resulting immune response may be dominated by the nitrophenyl and benzylic ring systems. Thus, antibodies generated will necessarily be anilidases and not amidases. NPN43C9 is, none the less, an important and interesting antibody in terms of the nitrogen leaving group in the reaction it catalyses and also because of the modelling and sequencing work carried out on it (*vide infra*).

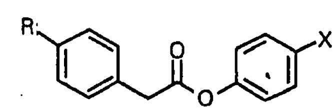
Bacterial expression

The total cDNA construct of NPN43C9 was expressed efficiently in *E. coli* cells and protein purified, and its catalytic properties were assessed in both the monoclonal antibody and the single-chain antibody (scFv) 7A4-1/212 for the hydrolysis of *p*-nitroanilide [50] and the related *p*-chlorophenyl ester [51a] (Fig. 19). Virtually identical k_{cat} and K_m values were obtained for both 7A4-1/212 and NPN43C9. This activity was decreased in both cases by the addition of the inhibitor *m*-nitroanilide [52], which gave $K_i = 800$ μM and 400 μM for the NPN43C9 and 7A4-1/212, respectively.

MECHANISTIC ANALYSIS

Kinetic analysis

NPN43C9 was shown to give a rate acceleration for hydrolysis of [50] of approximately 1.5×10^5, and its values of K_m and V_{max} were approximately the same as those for its Fab fragment, whose RNA sequence was subsequently used in cloning and expression of Fabs in a bacteriophage λ system (Huse *et al.*, 1989). Such an enterprise is capable of giving a greatly



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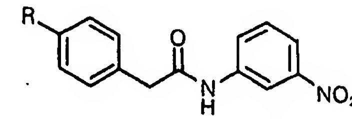


[52]

a X = Cl c X = CHO e X = H
b X = COCH$_3$ d X = CH$_3$ f X = NO$_2$

R = NHC(O)(CH$_2$)$_3$CO$_2$H

Fig. 19 Ester [51a] was used to investigate the comparative catalytic efficiency of the scFv 7A4-1/212 and the parent mAb NPN43C9. This activity was inhibited by *m*-nitroanilide [52].

expanded number of potential catalysts. It prompted a further study in which the coding sequences of the variable heavy (V_H) and variable light chain (V_L) fragments were used in the assembly of a single-chain antibody (Gibbs *et al.*, 1991).

The phosphonamidate [49] used to elicit 43C9 was designed to encourage general acid–base catalysis via oxyanion stabilization and protonation of the amide nitrogen in the tetrahedral transition state. However, results of pH–rate profiles in both D_2O and H_2O indicated that the mechanism involved an anionic transition state, probably progressing from the TI$^-$ (Benkovic *et al.*, 1990, 1991). The behaviour of the Michaelis–Menten parameters, k_{cat}/K_m and k_{cat} as a function of pH shows that catalytic activity increases with increasing pH to a maximum with an apparent pK_a of 9.0. Furthermore, the analysis helps to explain the deviation by almost 10^3 of the value of k_{cat}/k_{uncat} above that predicted on the basis of K_m/K_i (Section 4). Benkovic has postulated that this deviation may be a consequence of chemical catalytic processes (e.g. general acid–base or nucleophilic catalysis) being involved in the binding site for 43C9.

The occurrence of a kinetic isotope effect in the pH-dependent region but its absence in the plateau region has been interpreted as suggesting the existence of two chemically distinct processes. The k_{cat} value at pH > 9 correlates with the rate-limiting formation of an acyl-antibody intermediate, whilst at low pH there is hydroxide-mediated hydrolysis of this intermediate. Moreover, ^{18}O incorporation experiments showed that very little ^{18}O exchange occurs in the NPN43C9-catalysed reaction relative to the uncatalysed one, which is consistent with acyl-intermediate formation preventing exchange (Janda *et al.*, 1991a). The existence of a covalent acyl-antibody intermediate was further supported by analysis of the effects of a range of *p*-substituents on phenyl ester hydrolysis (Gibbs *et al.*, 1992a). The antibody was found to catalyse hydrolysis of less reactive substrates [51a–e] within a rate factor of 10 of that for the *p*-nitroester substrate [51f], indicating that breakdown of the intermediate is the rate-determining step.

Substrate variations

Analysis of the substituent effects on NPN43C9 catalysis was achieved using a Hammett σ–ρ correlation. A large ρ value of +2.3 was seen for the antibody-catalysed reaction. Such a large dependency on the leaving group is characteristic of nucleophilic attack by a neutral nitrogen nucleophile such as imidazole. By contrast, hydrolysis via general base catalysis would result in little charge build-up on the phenol oxygen and a low ρ value of 0.5–0.7 would be expected. Nucleophilic attack by, for example, hydroxide would lead to greater charge build-up in the $TS^{\ddagger}$ and a higher ρ value of ~1.0–1.2. That a histidine residue was the likely candidate for this nucleophilic role was pinpointed by two further experiments. First, chemical modification of NPN43C9 with a variety of reagents was inhibitory only with diethyl pyrocarbonate (DEPC), a reagent specific for histidine residues. Secondly, molecular modelling of the antibody binding site region highlighted two histidine residues, one of which was suitably positioned for attack on the substrate carbonyl group.

SITE-DIRECTED MUTAGENESIS AND COMPUTER MODELLING

The use of site-directed mutagenesis and computer modelling enabled the ligand binding and catalytic residues to be identified (Stewart *et al.*, 1994). A computer model of NPN43C9 with bound antigen identified specific residues as targets for site-specific mutagenesis, namely Tyr^{L32}, His^{L91}, Arg^{L96}, His^{H35} and Tyr^{H95}. Replacement of His^{L91} by a glutamine generated a mutant devoid of catalytic activity but with an affinity for the hapten almost as high as for the parent antibody. This implicated His^{L91} as the nucleophilic imidazole responsible for acyl-antibody intermediate formation. Arg^{L96} was also shown to be important for catalysis since, as predicted by modelling, its proximity to the carbonyl carbon suggested it should stabilize the anionic tetrahedral transition state. Mutation of Arg^{L96} to a neutral glutamine was found to destroy catalytic activity. Thus, the positively charged amino acid side-chain was assigned as flanking an oxyanion hole, polarizing the substrate carbonyl for nucleophilic attack, and stabilizing the anionic transition state by electrostatic interaction.

The resultant mechanism for the hydrolysis of a *p*-nitrophenyl ester substrate is as follows. Substrate binding orientates the guanidinium cation of Arg^{L96} towards the carbonyl group, locating the carbonyl carbon proximate to His^{L91}. Attack of an imidazole nitrogen of His^{L91} generates the acyl intermediate, assisted by coulombic interactions from Arg^{L96}. The breakdown of the acyl-antibody intermediate involves attack by hydroxide and sequential release of antibody followed by phenol and acid products (Fig. 20).

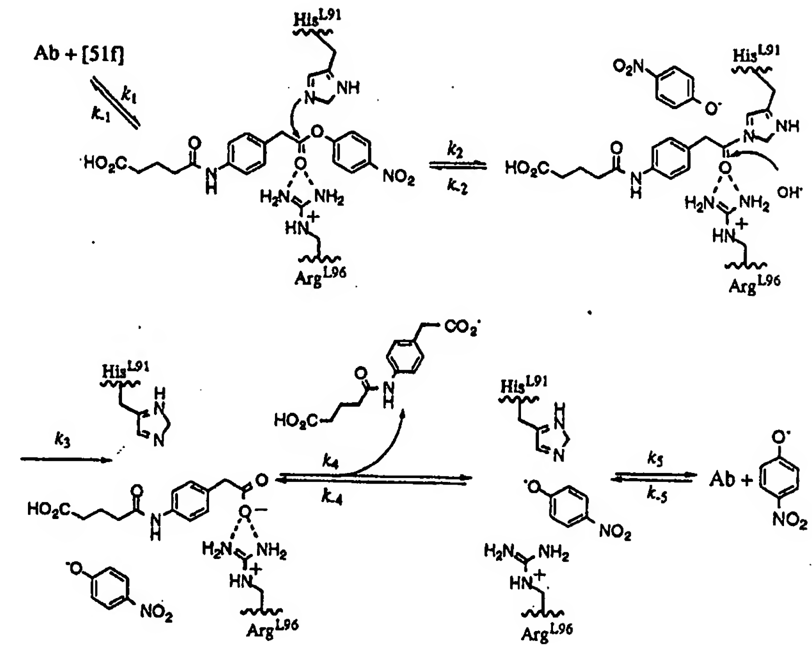


Fig. 20 The proposed catalytic mechanism for hydrolysis of ester substrate [51f] showing proposed roles for active site residues Arg^{L96} and His^{L91}.

In conclusion, NPN43C9 provides an excellent example of the application of standard techniques of physical organic chemistry in the characterization of an antibody both mechanistically and structurally.

6 Rescheduling the regio- and stereo-chemistry of parallel chemical reactions

The control of kinetic vs thermodynamic product formation can often be achieved by suitable modification of reaction conditions. A far more difficult task is to switch from the formation of a favoured major product to a disfavoured minor product, especially when the transition states for the two processes share most features in common. This challenge has been met by antibodies with considerable success, both for reaction pathways differing in regioselectivity and also for ones differing in stereoselectivity. In both situations, control of entropy in the transition state must hold the key.

DIELS–ALDER CYCLOADDITIONS

In the Diels–Alder reaction between an unsymmetrical diene and dienophile, up to eight stereoisomers can be formed (March, 1992a). It is known that the regioselectivity of the Diels–Alder reaction can be biased so that only the four *ortho*-adducts are produced (Fig. 21) through increasing the electron-withdrawing character of the substituent on the dienophile (Danishefsky and Hershenson, 1979). However, stereochemical control of the Diels–Alder reaction to yield the disfavoured *exo*-products in enantiomerically pure form has proved to be very difficult.

Gouverneur *et al.* (1993) were interested in controlling the outcome of the reaction between diene [53] and *N,N*-dimethylacrylamide [54] (Fig. 22). They had shown experimentally that the uncatalysed reaction gave only two

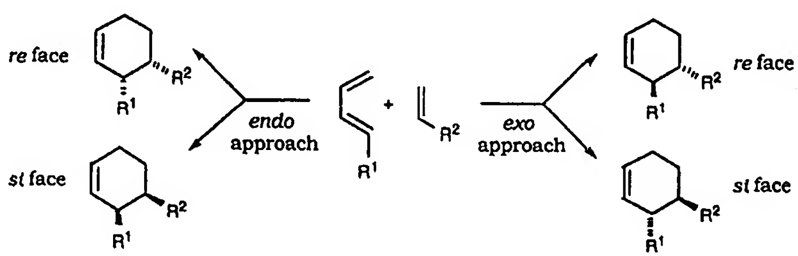
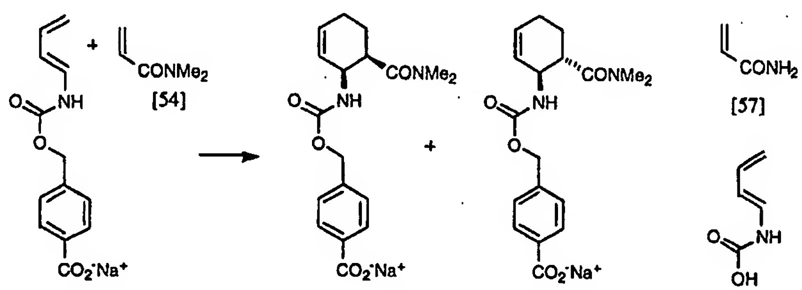


Fig. 21 Enantio- and diastereo-selectivity of the Diels–Alder reaction for *ortho*-approach.



[53] ± [55] ± [56] [58]

Fig. 22 The Diels–Alder cycloaddition between the dienophile [54] and diene [53] yields two diastereoisomers [55] and [56]. Attenuated substrate analogues [57] and [58] were used in molecular orbital calculations of this reaction.

Table 3 Calculated activation energies of the transition structures relative to reactants for the reaction of acrylamide [57] with *N*-(1-butadienyl)carbamic acid [58].

Transition state geometry	Calculated activation energy/kcal mol^{-1} RHF/3-21G	6-31G*/3-21G
Ortho-endo	27.3	40.80
Ortho-exo	28.84	42.70
Meta-endo	29.82	42.88
Meta-exo	30.95	43.94

stereoisomers; the *ortho-endo* (*cis*) [55] and the *ortho-exo* (*trans*) [56] adducts in an 85:15 mixture. This experimental observation was underpinned by *ab initio* transition state modelling for the reaction of acrylamide [57] with *N*-butadienylcarbamic acid [58], which showed that the relative activation energies of the *ortho-endo* and *ortho-exo* transition states were of considerably lower energy than the *meta-endo* and *meta-exo* transition structures (not illustrated) (Table 3).

The design of hapten was crucial for the generation of abzymes to deliver full regio- and diastereo-selectivity. Transition state analogues were thus devised to incorporate features compatible with either the disfavoured *endo* [59] or favoured *exo* [60] transition states (Fig. 23) (Appendix entry 17.5). Furthermore, because the transition state for Diels–Alder processes is very product-like, haptens [61] and [62] were developed to mimic a high-energy, boat conformation for each product, a strategy developed by Hilvert to minimize product binding to the abzyme (Hilvert *et al.*, 1989).

Two of the monoclonal antibodies produced, 7D4 and 22C8, proved to be completely stereoselective, separately catalysing the *endo* and the *exo* Diels–Alder reactions, with a k_{cat} of 3.44×10^{-3} and $3.17 \times 10^{-3}\ min^{-1}$ respectively at 25°C. That the turnover numbers are low was attributed in part to limitations in transition state representation: modelling studies had shown that the transition states for both the *exo* and *endo* processes were asynchronous whereas both TSAs [61] and [62] were based on synchronous transition states (Gouverneur *et al.*, 1993).

In a further enterprise, compounds [63] and [64] (Fig. 24) were perceived as freely rotating haptens for application as TSAs for the same Diels–Alder addition. As expected, each proved capable of inducing both *endo*- and *exo*-adduct-forming abzymes. It can be noted that [63] produced more "*exo*-catalysts" (6 out of 7) whereas [64] favoured the production of "*endo*-catalysts" (7 out of 8), though it is difficult to draw any conclusion from this observation (Appendix entry 17.5) (Yli-Kauhaluoma *et al.*, 1995).

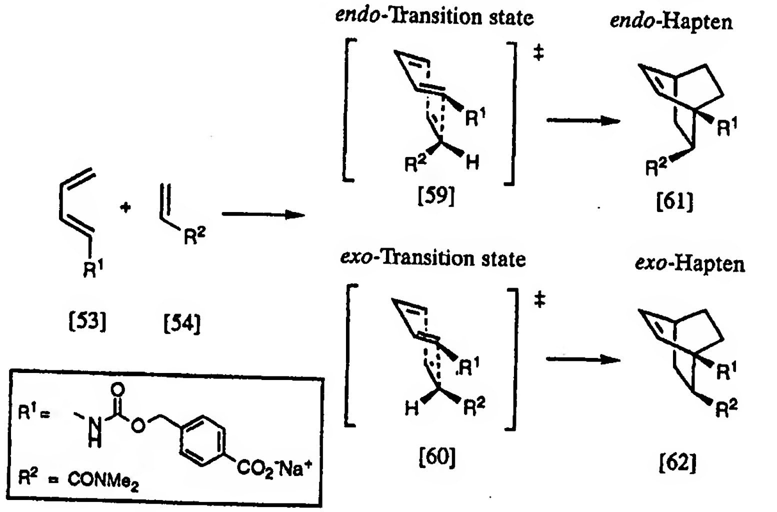


Fig. 23 Design of haptens [61] and [62], which are analogues of the favoured *endo*- [59] or disfavoured *exo*- [60] transition states, respectively.

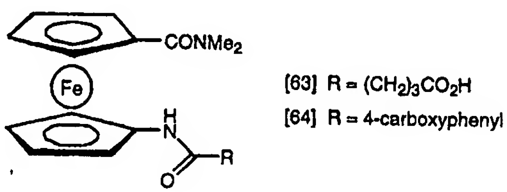


Fig. 24 An alternative strategy for eliciting Diels–Alderase antibodies has employed freely rotating ferrocenes [63] and [64] as TSAs.

DISFAVOURED REGIO- AND STEREO-SELECTIVITY

Reversal of kinetic control in a ring closure reaction

In reactions where several different outcomes are possible, the final product distribution reflects the relative free energies of each transition state when the reaction is under kinetic control (Schultz and Lerner, 1993). Baldwin's rules predict that for acid-catalysed ring closure of the hydroxyepoxide [65] the tetrahydrofuran product [66] arising from 5-*exo-tet* attack will be preferred

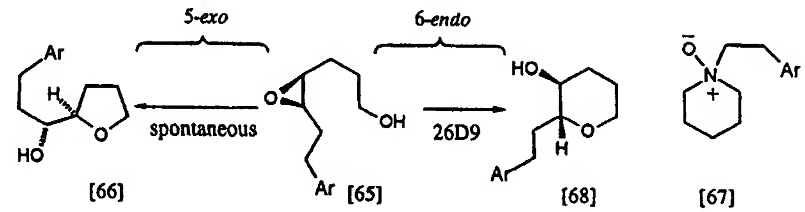


Fig. 25 The monoclonal antibody 26D9, generated to the *N*-oxide hapten [67], catalysed the 6-*exo-tet* ring closure of [65] regioselectively to yield the *disfavoured* tetrahydropyran product [68]. This is a formal violation of Baldwin's rules, which predicts a 5-*exo-tet* spontaneous process to generate tetrahydrofuran derivative [66].

(Fig. 25) (Baldwin, 1976). By raising antibodies to the charged hapten [67], Janda and co-workers produced an abzyme which accelerated 6-*exo* attack of the racemic epoxide to yield exclusively the *disfavoured* tetrahydropyran product [68] and in an enantiomerically pure form (Appendix entry 14.1) (Janda *et al.*, 1993).

This work reveals that an antibody can selectively deliver a single regio- and stereo-chemically defined product for a reaction in which multiple, alternative transition states are accessible and can also selectively lower the energy of the higher of two alternative transition states.

Syn-*elimination of β-fluoroketones*

The base-catalysed β-elimination of HF from the ketone [68] normally gives the favoured (*E*)-product [69] via a staggered conformation in the transition state. Hapten [70] is designed to enforce the *syn*-coplanar conformation of the phenyl and benzoyl functions in the transition state and so catalyse the disfavoured *syn*-elimination of [68] to give the (*Z*)-α,β-unsaturated ketone [71] (Fig. 26). Preliminary estimates of the energy difference between the favoured and disfavoured processes are close to 5 kcal mol^{-1} (Cravatt *et al.*, 1994), though this value is exceeded in the antibody-catalysed rerouting of carbamate hydrolysis from E1cB to $B_{Ac}2$ (Section 9, Appendix entry 5.3) (Wentworth *et al.*, 1997). Antibody 1D4, raised to hapten [70] and used in 15% DMSO at pH 9.0 and 37°C, gave exclusively the (*Z*)-product [71] with K_m 212 μM and k_{cat} 2.95×10^{-3} min^{-1}. Under the same conditions, k_{obs} is 2.48×10^{-4} min^{-1} for formation of [69] and immeasurably slow for the (undetectable) formation of [71] (Appendix entry 8.1).

CATIONIC CYCLIZATIONS

The cationic cyclization of polyenes to give multi-ring carbocyclic compounds with many sterically defined centres is one of the more remarkable examples

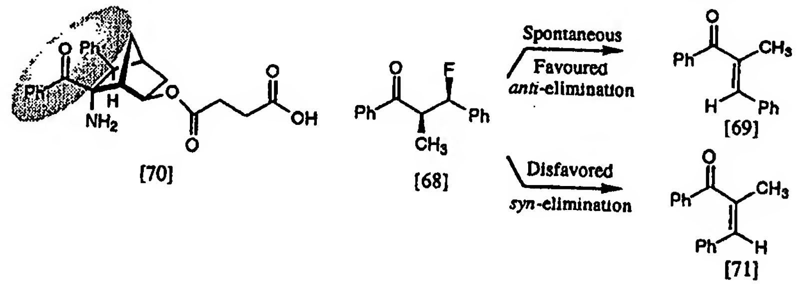


Fig. 26 The elimination of HF from the β-fluoroketone [68] is catalysed by antibody 1D4, elicited to hapten [70], to form the disfavoured (*Z*)-olefin [71]. This contrasts with the spontaneous process in which an *anti*-elimination reaction yields the (*E*)-α,β-unsaturated ketone [69]. The *syn*-eclipsed conformation of [70] is shaded.

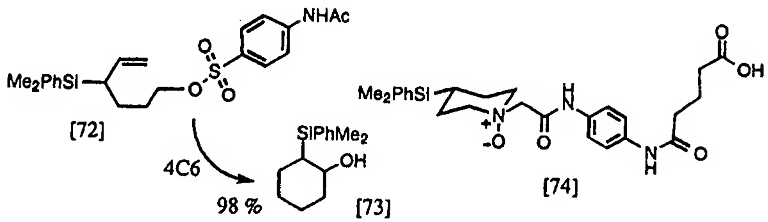


Fig. 27 The *N*-oxide hapten [74] was used to elicit mAb 6D4 which catalysed the cyclization of [72] to form the cyclohexanol [73].

of regioselective and stereoselective enzyme control which has provided a major challenge for biomimetic chemistry (Johnson, 1968). It provides an excellent opportunity for the application of regio- and stereo-control by catalytic antibodies.

Li *et al.* (1997) have discussed the use of catalytic antibodies to control the reactivity of carbocations. At an entry level, the acyclic olefinic sulfonate ester [72] is converted into the cyclic alcohol [73] (98%) using antibody 4C6 raised to hapten [73] with only 2% of cyclohexene produced (Appendix entry 15.1) (Li *et al.*, 1994).

Moving closer to a cationic transition state mimic, Hasserodt *et al.* (1996) used the amidinium ion [75] as a TSA for cyclization of the arenesulfonate ester [76]. One antibody raised to this hapten, 17G8, catalysed the conversion of substrate [76] into a mixture of the 1,6-dimethylcyclohexene [77] and 2-methylene-1-methylcyclohexane [78] (Fig. 28) (Appendix entry 15.3). By contrast, the uncatalysed cyclization of [76] formed a mixture of 1,2-

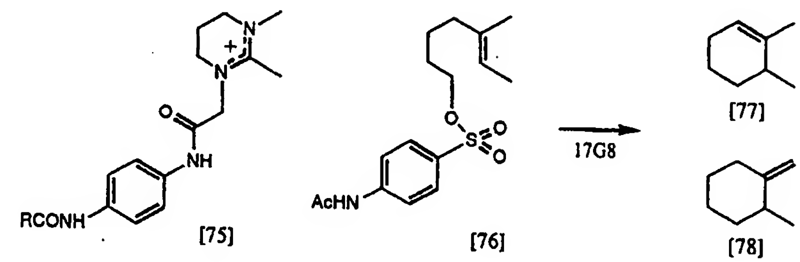


Fig. 28 Antibody 17G8 raised against TSA [75] catalysed the cyclization of [76] to give [77] and [78].

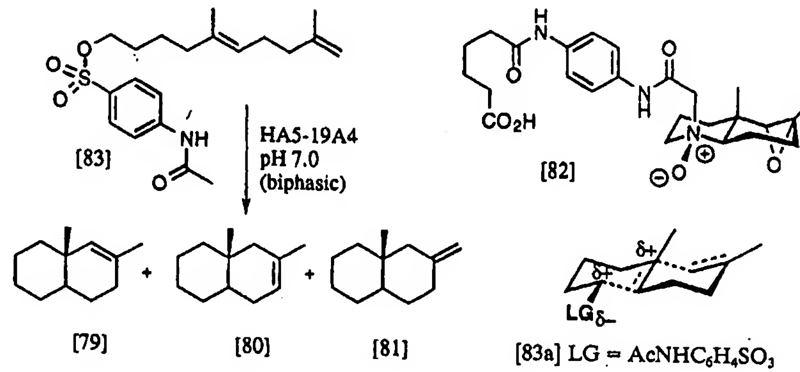


Fig. 29 Formation of isomeric decalins [71]–[73] by cyclization of a terpenoid alcohol catalysed by antibody HA5-19A4 raised to hapten [82]. The transition state [83a] has the leaving group in the equatorial position, as favoured by the Stork–Eschenmoser hypothesis.

dimethylcyclohexanols and a little 1,2-dimethylcyclohexene. Evidently, the antibody both excludes water from the transition state and also controls the loss of a proton following cyclization.

While cyclopentanes have also been produced by antibody-catalysed cyclization (Appendix entry 15.2) (Li *et al.*, 1996), much the most striking example of cationic cyclization by antibodies is the formation of the decalins [79], [80] and [81] (Fig. 29). The *trans*-decalin epoxide [82] ($t_{1/2}$ 100 h at 37°C) was employed as a mixture of two enantiomeric pairs of diastereoisomers as a TSA to raise antibodies, among which HA5-19A4 emerged as the best catalyst for cyclization of substrate [83] (Appendix entry 15.4) (Hasserodt *et al.*, 1997).

Sufficient substrate [83] was transformed to give 10 mg of mixed products.

The olefinic fraction (70%) was predominantly a mixture of the three decalins [79], [80] and [81] in a 2:3:1 ratio and formed along with a diastereoisomeric mixture of cyclohexanols (30%). Moreover, the decalins were produced with enantiomeric excesses of 53%, 53% and 80%, respectively. It is significant that the (*Z*)-isomer of [83] is not a substrate for this antibody.

Quite clearly, the antibody first catalyses ionization of the arenesulfonate to generate a carbocation. This process shows an ER of 3200 with K_m 320 μM. The resulting cation can then either cyclize to decalins in a concerted process (as in transition state [83a]) or in two stepwise cyclizations. The formation of significant amounts of cyclohexanols seems to indicate that the latter is the case. Most interestingly, inhibition studies strongly suggest that the isomer of the haptenic mixture that elicited this antibody has structure [82], which would locate the leaving group in an axial position. This is contrary to the Stork–Eschenmoser concept of equatorial leaving group and presents a challenge for future examination (Eschenmoser *et al.*, 1955; Stork and Burgstahler, 1955).

It is an exciting prospect that catalysts of this nature may lead to artificial enzymes capable of processing natural and unnatural polyisoprenoids to generate various useful terpenes.

7 Difficult processes

As exponents of catalytic antibodies have become more confident of the power of abzymes, their attention has turned from reactions of moderate to good feasibility to more demanding processes. Their work has on the one hand tackled more adventurous stereochemical problems and on the other hand is attempting to catalyse reactions whose spontaneous rates are very slow indeed. Examples of both of these areas are discussed in this section.

DIASTEREOISOMERIC RESOLUTION

Antibodies generally show very good recognition in favour of their antigens and against regio- or stereo-isomers of them. This results from a combination of the inherent chirality of proteins and the refined response of the immune system (Playfair, 1992). In extension, this character suggests that a catalytic antibody should be capable of similar discrimination in its choice of substrate and the transition state it can stabilize, as determined by the hapten used for its induction. As already shown above, the murine immune system can respond to a single member of a mixture of stereoisomers used for immunization (Section 6). Such discrimination has been exemplified in antibody-catalysed enantioselective ester hydrolysis (Janda *et al.*, 1989; Pollack *et al.*, 1989; Schultz, 1989) and transesterification reactions (Wirsching *et al.*, 1991; Jacobsen *et al.*, 1992).

Table 4 Kinetic parameters for those antibodies raised against phosphonates [88–91] which effect the resolution of the fluorinated alcohols [84–87]. The configuration of the disastereoisomerically pure product from each antibody-catalysed process was shown to correspond to that of the antibody-inducing hapten.

Alcohol product	Hapten	Configuration	$k_{cat}{}^a$/min^{-1}	K_m/μM	% ee/de of product
[84]	[88]	2*R*, 3*R* (+)	0.88	390	99.0
[85]	[89]	2*S*, 3*S* (−)	0.91	400	98.5
[86]	[90]	2*R*, 3*S* (+)	0.94	410	98.5
[87]	[91]	2*S*, 3*R* (−)	0.86	380	98.0

[a] At 25°C.

One study has made use of abzyme stereoselectivity to resolve the four stereoisomers (*R,R'*, *S,S'*, *R,S'* and *S,R'*) of 4-benzyloxy-3-fluoro-3-methylbutan-2-ol [84–87] through the antibody-mediated hydrolysis of a diastereoisomeric mixture of their phenacetyl esters (Kitazume *et al.*, 1991b). Antibodies were raised separately to each of four phosphonate diastereoisomers [88–91], corresponding to the four possible transition states for the hydrolysis of the four diastereoisomeric esters (Fig. 25) (Appendix entry 1.12). Each antibody operated on a mixture of equal parts of the four diastereoisomers as substrate to give each alcohol in ~23% yield, with >97% *ee/de*, and leaving the three other stereoisomers unchanged. By sequential action of the four antibodies in turn, the mixture of diastereoisomers could effectively be separated completely (Table 4). In a similar vein, Kitazume also resolved the enantiomers of 1,1,1-trifluorodecan-2-ol with 98.5% enantiomeric excess (Appendix entry 1.11) (Kitazume *et al.*, 1991a).

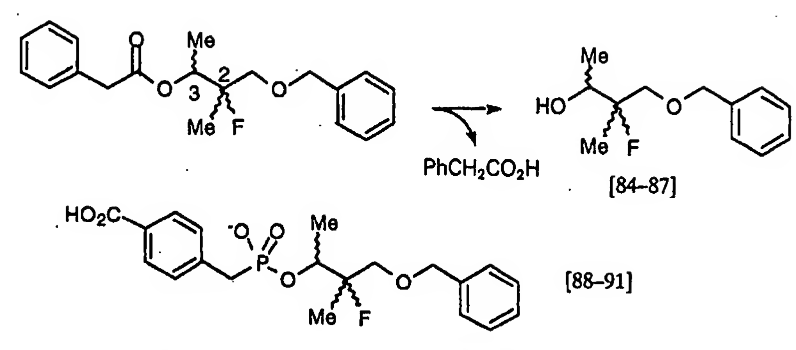


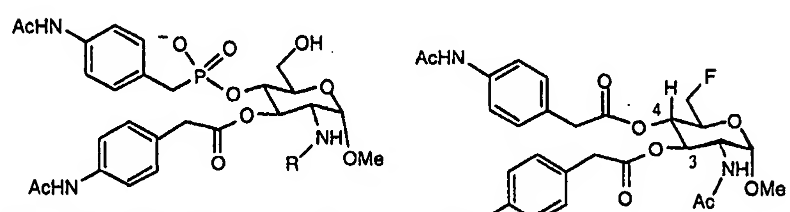
Fig. 30 Four stereoisomeric alcohols [84-87] were separated by selective hydrolysis of their respective phenacetyl esters using four antibody catalysts, each raised in response to a discrete stereoisomeric phosphonate hapten [88–91].

ACETAL AND GLYCOSIDE CLEAVAGE

Antibody-catalysed transformations for the synthesis, modification and degradation of carbohydrates are a subject of active investigation. Preliminary studies have reported antibody hydrolysis of model glycoside substrates (Yu *et al.*, 1994) while the regio- and stereo-selective deprotection of acylated carbohydrates has been achieved using abzymes with moderate rate enhancements. Antibodies raised against the TSA [92] were screened for their ability to hydrolyse the diester [93]. One antibody, 17E11, used in a 20% concentration with respect to [93], effected hydrolysis exclusively at C-4. This process was fast enough to render spontaneous C-3 to C-4 acyl migration insignificant: i.e. no C^3—OH product was detected. (This migration reaction is generally fast compared to chemical deacylation) (Fig. 31. Appendix entry 1.7) (Iwabuchi *et al.*, 1994). In this context, the use of an antibody to cleave a trityl ether by an S_N1 process may have further applications (Appendix entry 7.1) (Iverson *et al.*, 1990). Also, the objective of utilizing abzymes in the regioselective removal of a specified protecting group has been extended to show that an antibody esterase can have broad substrate tolerance (Appendix entry 1.18) (Li *et al.*, 1995b).

The assault on the demanding task of glycosylic bond cleavage is making good, albeit slow, progress. As a first step, Reymond has described an antibody capable of catalysing the acetal hydrolysis of a phenoxytetrahydropyran, though it is slow, with k_{cat} 7.8×10^{-5} s^{-1} at 24°C, and has a modest ER of 70 (Appendix entry 7.4B) (Reymond *et al.*, 1991).

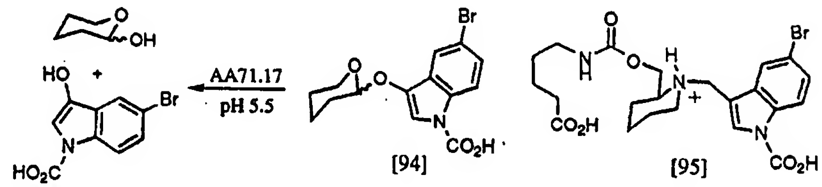
A general approach to the task has been to raise antibodies to TSAs related



R = $COCH_2CH_2SH$ [92] [93]

Abzyme identity	Conditions	K_m [93]	k_{cat} [93]	K_i [92]
17E11	pH 8.2; 20°C	6.6 μM	0.182 min^{-1}	0.026 μM

Fig. 31 Antibody 17E11 raised against the TSA [92] was screened for its ability to hydrolyse diester [93] and, used in a 20% concentration with respect to [93], effected hydrolysis exclusively at C-4.



[94] [95]

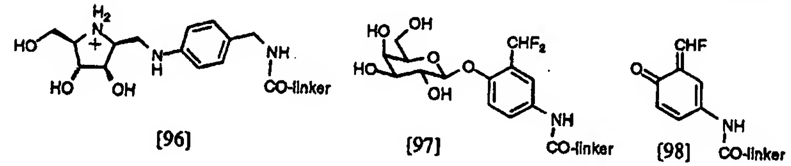
Fig. 32 Antibody AA71.17 raised against hapten [95] catalyses the hydrolysis of the aryl acetal [94].

by design to well-known inhibitors of glycosidases. Piperidino and pyrrolidino cations have high affinity for pyranosidases and furanosidases (Winchester and Fleet, 1992; Winchester *et al.*, 1993) and can also be envisaged as components of a "bait and switch" approach to antibody production. Thus, Schultz has described the hydrolysis of the 3-indolyl acetal [94] (Fig. 32) by antibody AA71.17 raised to transition state analogue [95] (Appendix entry 7.2) (Yu *et al.*, 1994). Antibody AA71.17 has a good K_m of 320 μM but is rather slow in turnover, k_{cat} 0.015 min^{-1} at 25°C. (By contrast, two other haptens based on a guanidino and a dihydropyran inhibitor did not elicit any antibodies that showed glycosidase activity.)

More recently, Janda has described the production of a galactopyranosidase antibody in response to hapten [96]. This was designed to accommodate several features of the transition state for glycoside hydrolysis: notably a flattened half-chair conformation and substantial sp^2 character at the anomeric position. Some 100 clones were isolated in response to immunization with [96] and used to generate a cDNA library for display on the surface of phage (Appendix entry 7.3) (Janda *et al.*, 1997). Rather than proceed to the normal screening for turnover, Janda then created a suicide substrate system to trap the catalytic species.

Halazy had earlier shown that phenols with *o*- or *p*-difluoromethyl substituents spontaneously eliminate HF to form quinonemethides that are powerful electrophiles and that this activity can be used to trap glycosidases (Halazy *et al.*, 1992). So, glycosylic bond cleavage in [97] (Fig. 33) results in formation of the quinonemethide [98] that covalently traps the antibody catalyst. By suitable engineering of a bacteriophage system, Janda was able to screen a large library of Fab fragment antibodies and select for catalysis. Fab 1B catalysed the hydrolysis at 37°C of *p*-nitrophenyl β-galactopyranoside with $k_{cat} = 0.007$ min^{-1} and $K_m = 530$ μM, corresponding to a rate enhancement of 70 000. Moreover, this activity was inhibited by hapten [96] with $K_i = 15$ μM. By contrast, the best catalytic antibody, 1F4, generated from hapten [96] by classical hybridoma screening showed $k_{cat} = 10^{-5}$ min^{-1} and $K_m = 330$ μM, a rate enhancement of only 100.

Clearly, this work both offers an exciting method for screening for



[96] [97] [98]

Fig. 33 Fragment antibody Fab1B is selected by suicide selection with substrate [97] from a library of antibodies generated to hapten [96]. The suicide intermediate is the *o*-quinonemethide [98].

antibodies that can lead to suicide product trapping and also appears to offer a general approach to antibodies with glycosidase activity.

PHOSPHATE ESTER CLEAVAGE

The mechanisms of phosphate ester cleavage vary significantly between monoesters, diesters, and triesters (Thatcher and Kluger, 1989). Each of these is a target for antibody cleavage and progress has been reported for all three cases.

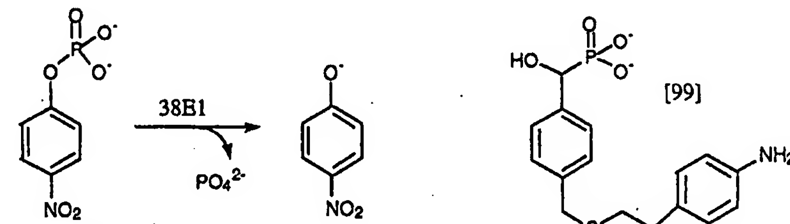
Phosphate monoesters

This reaction is a particular challenge in light of the fact that phosphoryl transfers involving tyrosine, serine or threonine play crucial roles in signal transduction pathways that are control elements of many aspects of cellular physiology. The generation of an abzyme would provide an important biological tool for the investigation and manipulation of such processes.

Schultz's group employed an α-hydroxyphosphonate hapten [99] and subsequently isolated 20 cell lines of which 5 catalysed the hydrolysis of the model substrate *p*-nitrophenyl phosphate [100] above background (Fig. 34) (Scanlan *et al.*, 1991). Antibody 38E1 was characterized in more detail and kinetic parameters were afforded by Lineweaver–Burke analysis. This antibody exhibited 11 turnovers per binding site with no change in V_{max}, and thus acted as a true catalyst. Moreover, examination of substrate specificity showed that catalysis was entirely selective for *p*-substituted species (Appendix entry 6.6).

Phosphodiester cleavage

Considering that the phosphodiester bond is one of the most stable chemical linkages in nature, its cleavage is an obvious and challenging target for antibody catalysis. In an attempt to model a metal-independent mechanism, a nucleotide analogue [101] comprising an *O*-phosphorylated hydroxylamine moiety was chosen by Sakurai and co-workers (Sakurai *et al.*, 1996).

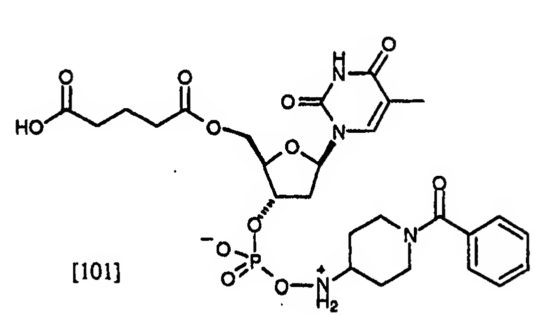


[100] [99]

Abzyme identity	Conditions	K_m [100]	$k_{cat}{}^{a}$ [100]	K_i [99]
38E1	pH 9.0	155 μM	0.0012 min^{-1}	34 μM

[a] At 30°C.

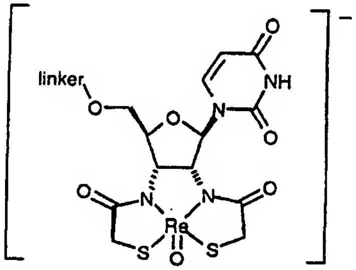
Fig. 34 (above) Antibody 38E1, generated from the α-hydroxyphosphonate hapten [99], catalysed the hydrolysis of *p*-nitrophenyl phosphate [100].



[101]

This hapten design aims to represent the geometry and spatial constraints in the phosphate linkage so as to retain the stereoelectronic configuration of the phosphorus atom, and finally to act as a simple model of a dinucleotide. To this end, the retention of the phosphate backbone seeks to facilitate the formation of an oxyanion hole in which the electrophilicity of the phosphorus centre is increased in the bound substrate, whilst the positive charge on the hapten is designed to elicit an anionic amino acid in the abzyme combining site, to act either as a general base to activate a nucleophilic water molecule or as a nucleophile operating directly at the phosphorus centre. More details are awaited from this work.

The classic case of assisted hydrolysis of phosphate diesters is neighbouring



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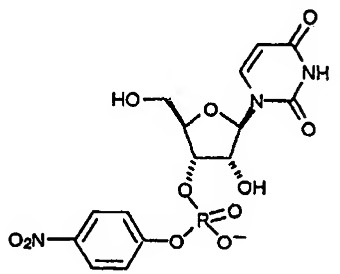


[103]

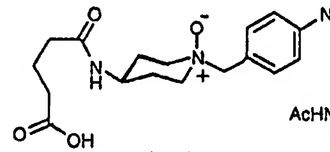
Fig. 35 Hapten *anti*-[102] was used to generate 25 mAbs from which 2G12 proved to catalyse the hydrolysis of the phosphate diester [103].

group participation by a vicinal hydroxyl group, specifically the phosphate ester of a 1,2-diol, which provides a rate acceleration greatly in excess of 10^6 (Westheimer, 1968). While vanadate complexes of 1,2-diols have been explored as pentacoordinate species for inhibiting enzymes, they are toxic and are too labile for use in murine immunization (Crans *et al.*, 1991). Janda has found a solution to this problem through the use of pentacoordinate oxorhenium chelates (Weiner *et al.*, 1997). Hapten [102] was employed as separate diastereoisomers to generate 50 monoclonal antibodies which were screened for their ability to hydrolyse uridine 3′-(*p*-nitrophenyl phosphate) [103] (Fig. 35) (Appendix entry 6.4). The most active of three antibodies, 2G12, had k_{cat} $1.53 \times 10^{-3}\,s^{-1}$ at 25°C and K_m 240 μM, giving k_{cat}/k_{uncat} 312. A more favourable expression for protein catalysts working at substrate concentrations below K_m is $k_{cat}/(K_m \times k_{uncat})$ (Radzicka and Wolfenden, 1995), which is $1.3 \times 10^6\,M^{-1}$ and compares favourably to the value for RNase A of $10^{10}\,M^{-1}$ for the same substrate. The TSA *anti*-[102] proved to be a powerful inhibitor for 2G12 with K_i estimated at 400 nM.

Evidently, this is a system with scope alike for improvement in design and for broader application. It is clearly one of the most successful examples of antibody catalysis of a difficult reaction.

Phosphotriester hydrolysis

Catalysis of this reaction was first exhibited by antibodies raised by Rosenblum *et al.* (1995). More recently, Lavey and Janda (1996a) have explored the generation of abzymes capable of catalysing the breakdown of poisonous agrochemicals. Twenty-five mAbs were raised against the *N*-oxide hapten [104] of which two were found to be catalytic. The hapten was designed to generate antibodies for the hydrolysis of triester [105] using the "bait and



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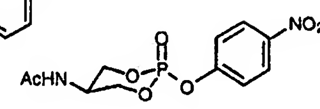

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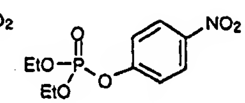


[106]

Abzyme identity	Substrate	Conditions	K_m	k_{cat}[a]	K_i [104]
15C5	[105]	pH 8.1, 25°C	87 μM	0.0027 min^{-1}	–
3H5	[106]	pH 9.15	5.05 mM	0.0020 min^{-1}	0.98 μM

[a]Measured at pH 8.25 and 25°C.

Fig. 36 The *N*-oxide [104] was used as hapten to raise mAbs to catalyse the hydrolysis of both triesters [105] and [106].

switch" methodology: cationic charge on the nitrogen atom targeted to induce anionic amino acids to act as general base catalysts; partial negative charge on oxygen to encourage the selection of antibody residues capable of stabilizing negative charge in the transition state (Fig. 36).

Antibody 15C5 was able to catalyse the hydrolysis of the triester [105] with k_{cat} $2.65 \times 10^{-3}\,min^{-1}$ whilst a second antibody from the same immunization programme was later found to hydrolyse the acetylcholinesterase inhibitor Paraoxon [106] with $k_{cat} = 1.95 \times 10^{-3}\,min^{-1}$ at 25°C (Appendix entry 6.2) (Lavey and Janda, 1996b). Antibody 3H5 showed Michaelis–Menten kinetics and was strongly inhibited by the hapten [104]. It exhibited a linear dependence of the rate of hydrolysis on hydroxide ion concentration, suggesting that 3H5 effects catalysis by transition state stabilization rather than by general acid/base catalysis.

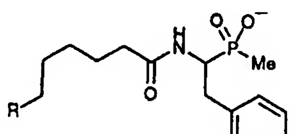
Phosphate ester hydrolysis is one of the most demanding of reactions for catalyst engineering. The progress made so far with catalytic antibodies is highly promising and appears to be competitive with studies using metal complexes if only because they can deliver *turnover* while metal complexes have for the most part to solve the problem of tight product binding.

AMIDE HYDROLYSIS

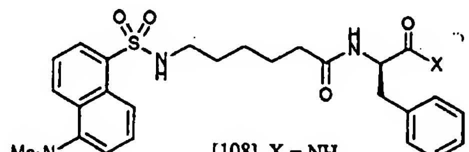
While ester, carbonate, carbamate and anilide hydrolyses have been catalysed effectively by antibodies, the difficult tasks of hydrolysis of an aliphatic amide or a urea remain largely unsolved. Much of this problem hinges on the fact that breakdown of a $TI^{\pm}$ is the rate-determining step, as established by much

kinetic analysis and, more recently, by computation. Teraishi has computed that C—N bond cleavage for the TI^- for hydrolysis of *N*-methylacetamide (or aminolysis of acetic acid) lies some 18 kcal mol^{-1} above that for C—O(H) bond cleavage (Teraishi *et al.*, 1992). Clearly, protonation of nitrogen has to be an essential feature of the breakdown of such intermediates and for anilides that is hardly a practical proposition under ambient conditions. To date only two investigations of this problem have shown any success.

A group at IGEN raised antibodies to the dialkylphosphinic acid [107]. These were screened for their ability to hydrolyse four alkyl esters and four primary amides at pH 5.0, 7.0, and 9.0. Just one out of 68 antibodies, 13D11, hydrolysed the C-terminal carboxamide stereospecifically of only the (*R*)-substrate [108], which was rendered visible by the attachment of a dansyl



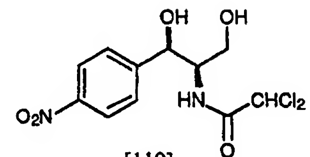
± [107]



[108] X = NH_2
[109] X = OMe

fluorophore to support hplc analysis of the course of the reaction (Appendix entry 5.1) (Martin *et al.*, 1994). At pH 9.0 and 37°C, 13D11 showed K_m 432 μM and k_{cat} $1.65 \times 10^{-7}\ s^{-1}$, a half-life of 42 d. This activity was fully inhibited by hapten [107] with $K_i = 14\ \mu$M. Unexpectedly, the dansyl group proved to be an essential component of the substrate. Even more unexpectedly the antibody did not hydrolyse the corresponding methyl ester [109].

Whereas most amide substrates for catalytic antibodies have been activated by the use of aromatic amines (Appendix entries 5.3, 5.4), Blackburn chose to explore hydrolysis of an aliphatic amide, activated through halogenation in the acyl moiety (Shen, 1995; Datta *et al.*, 1996). Chloramphenicol [110] was selected as substrate on account of its dichloroacetamide function and the tetrahedral intermediate for hydrolysis was mimicked by the neutral sulfonamide [111] and the zwitterionic "stretched transition state analogue" aminophosphinic acid [112]. Antibodies produced to each of these haptens proved too weak to hydrolyse chloramphenicol at a rate sufficiently above background ($k_{OH} = 1.3 \times 10^{-3}\ M^{-1}\ s^{-1}$) for further study. However, a switch to the more active amide, trifluoramphenicol [113] ($k_w = 6 \times 10^{-7}\ s^{-1}$, $k_{OH} = 6.3 \times 10^{-2}\ M^{-1}\ s^{-1}$ at 37°C), enabled useful data to be obtained for antibody 2B5 which showed Michaelis–Menten kinetics with $k_{cat} = 2 \times 10^{-6}\ s^{-1}$ and $K_m = 640\ \mu$M at pH 7.0, 37°C. Once again, the use of $k_{cat}/(K_m \times k_{uncat})$ gives a more favourable value for the ER of 5200. The high K_m is likely to be a consequence of exchanging the dichloroacetamide moiety



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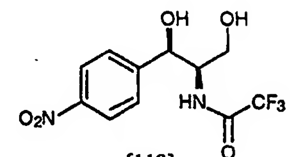
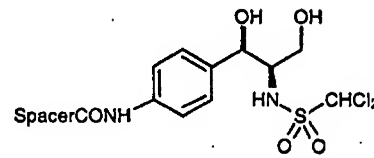


[113]



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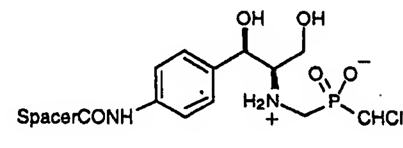


[112]

in the hapten for the trifluoroacetamide group in the substrate and could presumably be improved by redesign of the hapten. The low rate of turnover achieved clearly indicates the difficult task ahead for antibody cleavage of a peptide based on tetrahedral intermediate mimicry alone.

By contrast, the reverse reaction, that of amide synthesis, has proved to be a good target for antibody catalysis and a range of different enterprises have been successful (Appendix entries 18.1–18.4). It would appear here that little more is needed than a good leaving group and satisfactory design of a TSA based on an anionic tetrahedral intermediate (Benkovic *et al.*, 1988; Janda *et al.*, 1988a; Hirschmann *et al.*, 1994; Jacobsen and Schultz, 1994).

8 Reactive immunization

A novel approach for the induction of catalysis in antibody binding sites is a strategy dubbed "reactive immunization" (Wirsching *et al.*, 1995). This system uses haptens of intermediate chemical stability as immunogens. After the first stimulation of the mouse B cells to generate antibodies, one of the products of *in vivo* chemical transformation of the original hapten is then designed to act as a second immunogen to stimulate further mutational development of antibodies that will be better able to catalyse the desired reaction. The system seems well designed to achieve the benefits of a neutral and a charged hapten within the same family of monoclonal antibodies.

An organophosphate diester [114], was chosen as the primary reactive immunogen. Following spontaneous hydrolysis *in vivo* it becomes a stable monoester transition state analogue [115], which in turn gives a new challenge to the immune system (Fig. 37) (Appendix entry 2.14). Cross-reactivity has been established as an advantage in this process since heterologous

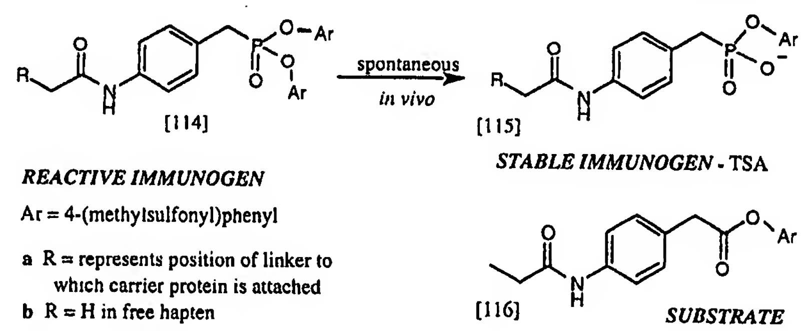


Fig. 37 Antibodies raised simultaneously against the reactive and stable immunogen shown above were capable of efficient "turnover" of the related aryl ester substrate [116] (Ab 49H4: $K_m = 300\ \mu M$; $k_{cat} = 31\ min^{-1}$ at 22°C).

immunization with both diaryl ester [114] and the corresponding monoaryl ester gave cross-reacting serum with enhanced affinity for the monoaryl TSA. This further promotes the induction of active-site amino acids capable of acting as nucleophiles or general acid/base catalysts. In practice, reactive immunization with [114a] generated 19 mAbs, 11 of which were able to hydrolyse substrate [114b]. The most efficient abzyme, SPO49H4, was analysed kinetically using radioactive substrates. It was established that 49H4 had undergone reactive immunization, since it was able to turn over the aryl carboxylate aryl [116] very effectively with $K_m = 300\ \mu M$; $k_{cat} = 31\ min^{-1}$.

A similar approach has been used by Lerner and Barbas to induce catalytic antibodies mimicking type I aldolases. The reaction scheme is shown in Fig. 38: the aim here was to induce an enamine moiety which can achieve catalysis through lowering the entropy for bimolecular reaction between ketone substrate and aldol acceptor. Compound [117] is a 1,3-diketone which acts to trap the "critical lysine", forming the vinylogous amide [118], which can be monitored spectrophotometrically at 318 nm (Appendix entry 16.2) (Lerner and Barbas, 1996). Screening for this catalytic intermediate by incubation with hapten facilitated the detection of two monoclonal antibodies with $k_{cat} = 2.28 \times 10^{-7}\ M^{-1}\ min^{-1}$. Furthermore, $k_{cat}/(K_m \times k_{uncat})$ is close to 10^9, making these antibodies nearly as efficient as the naturally occurring fructose 1,6-bisphosphate aldolase. Studies on the stoichiometry of the reaction by titration of antibody with acetylacetone indicated two binding sites to be involved in the reaction.

The antibodies generated in this programme were initially found to accept a broad range of substrates including acetone, fluoroacetone, 2-butanone, 3-pentanone and dihydroxyacetone. The list has now been expanded to include

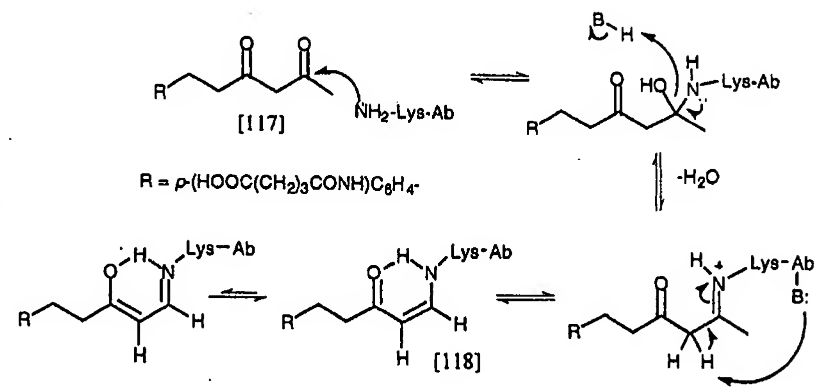
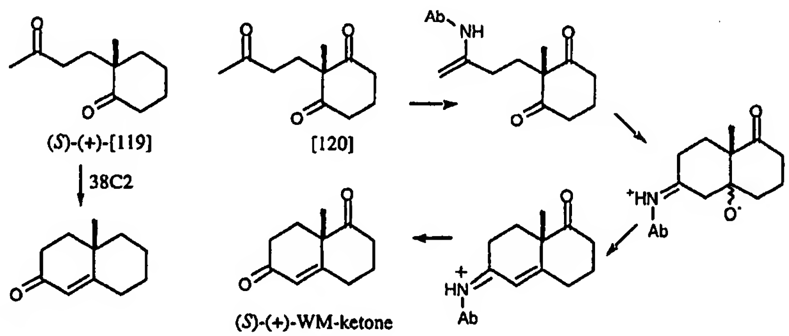


Fig. 38 The mechanism by which an essential Lys residue in the antibody combining site is trapped using the 1,3-diketone [117] to form the covalently linked vinylogous amide [118].

hundreds of different aldol condensations. However, a more remarkable property of 38C2 emerged when it was screened for its ability to catalyse an intramolecular Robinson annulation reaction (Fig. 39). Ab38C2 accepts equally well both the (*R*)-(−) and (*S*)-(+) enantiomers of 2-(3-oxobutyl)-2-methylcyclohexanone [119] and converts them stereospecifically into the respective stereoisomer of 1-methyldecal-5-en-3-one: (*R*)-isomer $k_{cat} = 0.126\ min^{-1}$, $K_m = 2.45$ mM; (*S*)-isomer $k_{cat} = 0.186\ min^{-1}$, $K_m = 12.4$ mM at 25°C (Appendix entry 16.2) (Zhong *et al.*, 1997).

While this example of the Robinson annulation is clearly not enantioselective, the same antibody converts the *meso*-ketone [120] into the Wieland–Miescher (WM) decalenedione product: $k_{cat} = 0.086\ min^{-1}$ and $K_m = 2.34$ mM at 25°C, parameters that give an impressive ER of 3.6×10^6. Good evidence suggests that the mechanism of the reaction involves the formation of a ketimine with the ε-amino group of a buried lysine residue in the antibody, as shown in Fig. 39. Most significantly, the reaction delivers the (*S*)-(+)-WM product in 96% *ee* (by polarimetry) and in 95% *ee* by nmr and hplc analysis for a 100 mg scale reaction. A recent report tells that this antibody is to be made commercially available at a cost of $100 for 10 mg. The realization of that objective would mark the start of a new era of application of abzymes to organic stereoselective synthesis.

Finally, the whole process of reactive immunization opens up the opportunity of using mechanism-based inhibitors as haptens, capable of actively promoting a desired mechanism by contrast to their conventional use as irreversible enzyme inhibitors.



Substrate	k_{cat}/min^{-1}	K_m/mM	k_{uncat}/min^{-1}	ER
(S)-(+)-[119]	0.186	12.4	nd	nd
(R)-(−)-[119]	0.126	2.45	nd	nd
[120]	0.086	2.34	2.4×10^{-6}	3.6×10^{6}

Fig. 39 Robinson annulation of cyclohexanones [119] and [120] catalysed by antibody Ab38C2 (Zhong *et al.*, 1997).

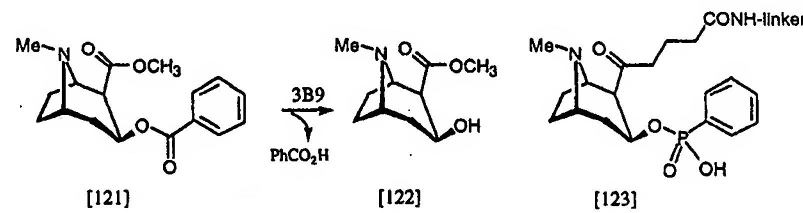
9 Medical potential of abzymes

DETOXIFICATION BY CATALYTIC ANTIBODIES

The idea that abzymes might be used therapeutically to degrade harmful chemicals *in homo* offers a new route to the treatment of victims of drug overdose. Landry's group have produced antibodies to catalyse the hydrolysis of the benzoyl ester of cocaine [121] yielding the ecgonine methyl ester [122] and benzoic acid, products which retain none of the stimulant or reinforcing properties of the parent drugs. The transition state for this cleavage was mimicked by the stable phosphonate monoester [123] which led to a range of antibodies of which 3B9 and 15A10 were the most effective (Fig. 40) (Appendix entry 1.3) (Landry *et al.*, 1993).

PRODRUG ACTIVATION BY CATALYTIC ANTIBODIES

Many therapeutic agents are administered in a chemically modified form to improve features such as their solubility characteristics, ease of administration and bioavailability (Bowman and Rand, 1988). Such a "prodrug" must be designed to break down *in vivo* to release the active drug, sometimes at a



Abzyme identity	Conditions	K_m [121]	k_{cat}[a] [121]	K_i [123]
3B9	pH 7.7	490 μM	0.11 min^{-1}	<2 μM
15A10	pH 8.0	220 μM	2.3 min^{-1}	–

[a]Temperature not defined.

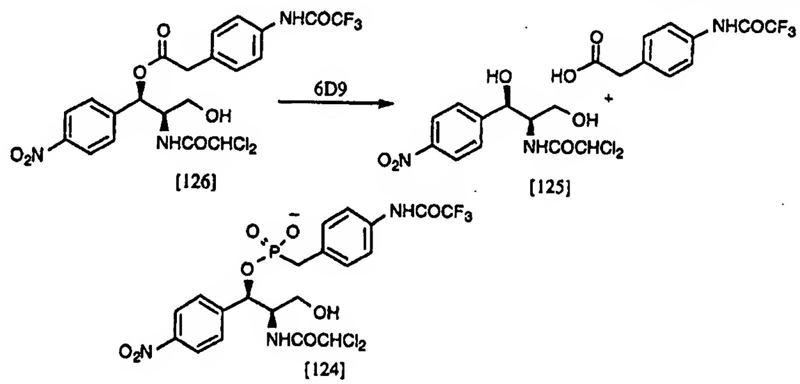
Fig. 40 Hapten [123] was used to raise an antibody 3B9 capable of the hydrolysis of cocaine [121] to the alcohol [122] thereby effecting cocaine detoxification.

particular stage of metabolism or in a particular organ. The limitation that this imposes on the choice of masking function could be overcome by the use of an antibody catalyst for unmasking the prodrug which could, in principle, be concentrated at a specified locus in the body. Such selectivity could have implications in targeted therapies.

Antibody-mediated prodrug activation was first illustrated by Fujii's group using antibodies raised against phosphonate [124] to hydrolyse a prodrug of chloramphenicol [125] (Fig. 41). Antibody 6D9 was shown to operate on substrate [126] to release the antibiotic [125] with an ER of 1.8×10^3 (Appendix entry 1.8) (Miyashita *et al.*, 1993). Furthermore, Fujii showed unequivocally that antibody-catalysed prodrug activation is viable by demonstrating inhibition of the growth of *B. subtilis* by means of the ester [126] only when antibody Mab 6D9 was present in the cell culture medium. The antibody-catalysed hydrolysis was unaffected by chloramphenicol at 10 mM and thus did not suffer from product inhibition, supporting the multiple turnover effect seen in the growth inhibition assay.

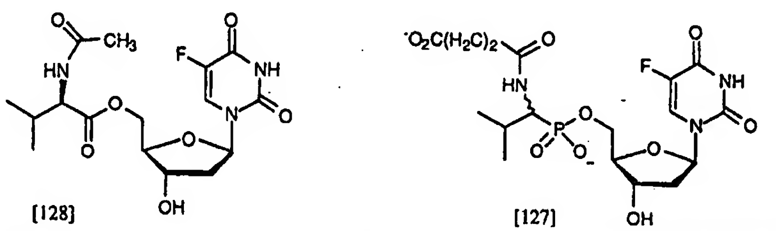
Campbell and co-workers also succeeded with this type of strategy by eliciting antibody 49.AG.659.12 against a phosphonate TSA [127], designed to promote release of the anti-cancer drug 5-fluorodeoxyuridine from a D-valyl ester prodrug [128] (Fig. 42) (Appendix entry 1.10) (Campbell *et al.*, 1994). This catalyst was able to bring about inhibition of the growth of *E. coli* by the release of the cytotoxic agent 5-fluorodeoxyuridine *in vitro*.

Much the most developed example of prodrug activation comes from our own laboratory. The cytotoxicity of nitrogen mustards is dependent on substitution on the nitrogen atom: electron-withdrawing substituents



Abzyme identity	Conditions	K_m [126]	k_{cat} [126]	K_i [124]
6D9	pH 8.0; 30°C	64 μM	0.133 min^{-1}	0.06 μM

Fig. 41 The monoclonal, 6D9, raised against phosphonate [124] catalysed the hydrolysis of one possible regio-isomer [125] of a phenacetyl ester prodrug derived from chloramphenicol [126].



Abzyme identity	Conditions	K_m [128]	k_{cat} [128]	K_i [127]
49.AG.659.12	pH 8.0; 37°C	218 μM	0.03 min^{-1}	0.27 μM

Fig. 42 Prodrug [128] is an acylated derivative of the anticancer drug 5-fluorodeoxyuridine. Antibody 49.AG.659.12, raised against phosphonate [127] was found to activate the prodrug [128] *in vitro*, thereby inhibiting the growth of *E. coli.*

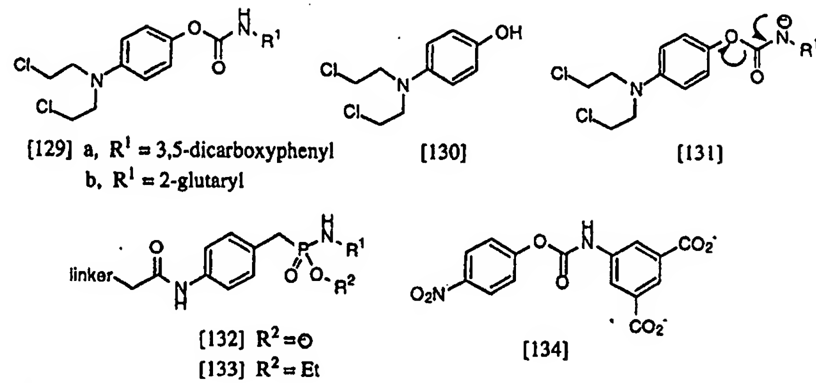


Fig. 43 Carbamate prodrugs [129a,b] are targets for abzyme cleavage to release a mustard [130] of enhanced cytotoxicity. E1cB hydrolysis of aryl carbonates involves the anion [131]. TSAs [132] and [133] were used to generate antibodies to catalyse a $B_{Ac}2$ mechanism for hydrolysis whose kinetic behaviour was evaluated with ester [134].

deactivate and electron-releasing substituents activate a bifunctional mustard. Thus, cleavage of a carbamate ester of a phenolic mustard can enhance its cytotoxicity to establish the carbamate as a viable prodrug for cancer chemotherapy (Blakey, 1992; Blakey *et al.*, 1995). So the target for prodrug activation is defined as an aryl carbamate whose nitrogen substituent is either an aryl [129a] or alkyl [129b] moiety.

Aryl carbamates are known to cleave by an E1cB process with a high dependency on the pK_a of the leaving phenol ($\rho^- = 2.5$). By contrast, aryl *N*-methylcarbamates are hydrolysed by a $B_{Ac}2$ process with a much lower dependency on leaving group ($\rho^0 = 0.8$) (Williams and Douglas, 1972a,b). Given the electron-releasing nature of the nitrogen mustard function ($\sigma \sim -0.5$), the kinetic advantage of antibody hydrolysis via the $B_{Ac}2$ pathway coupled to the proven ability of antibodies to stabilize tetrahedral transition states led to the formulation of TSAs [132] and [133]. Siting the linker in the locus of the nitrogen mustard was designed (i) to minimize potential alkylation of the antibody by the mustard function and (ii) to support mechanistic investigations by variation of the *p*-substituent of the aryl carbamate with little or no change in K_m, both features that were realized in the outcome. Both of these TSAs generated large numbers of hybridomas including many catalysts capable of carbamate hydrolysis.

A mechanistic analysis of antibody DF8-D5 showed it to cleave *p*-nitrophenyl carbamate [134] with $k_{cat} = 0.3\ s^{-1}$, $K_m = 120\ \mu M$, and $k_{cat}/k_{uncat} = 300$ at 14°C (Appendix entry 4.3) (Wentworth *et al.*, 1997). This is some tenfold more active than a carbamatase antibody generated by Schultz

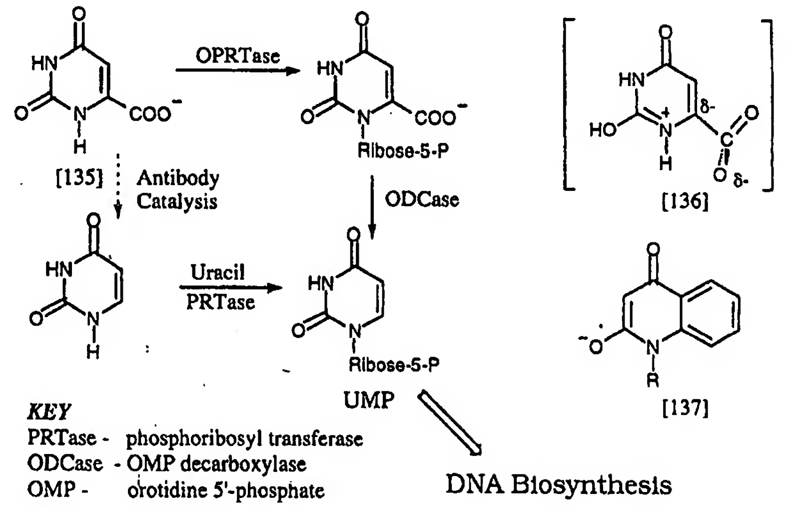
to a *p*-nitrophenyl phosphonate TSA but with a similar ER (Appendix entry 4.1) (Van Vranken *et al.*, 1994). Most significantly, variations in the *p*-substituent in substrates for DF8-5 hydrolysis identified a Hammett ρ^0 value[5] of 0.526 to establish the $B_{Ac}2$ nature of the reaction. For the *p*-methoxyphenyl carbamate substrate ($\sigma^0 = -0.3$) the apparent ER is 1.2×10^6. Given that there is a 10^8 difference in rate for the E1cB and $B_{Ac}2$ processes for the *p*-nitrophenyl carbamate [134], the data show that antibody DF8-D5 has promoted the disfavoured $B_{Ac}2$ process relative to the spontaneous E1cB cleavage by some 13 kcal mol^{-1}. Lastly, it is noteworthy that DF8-D5 was raised against the phosphonate diester [133a] as hapten, which raises the possibility that it may be an unexpected product of reactive immunization (Section 8).

The medical potential of such carbamatases depends on their ability to deliver sufficient cytotoxic agent to kill cells. Antibody EA11-D7, raised against TSA [133b] proved able to hydrolyse the prodrug [129b] with $K_m = 201\ \mu M$ and $k_{cat} = 1.88\ min^{-1}$ at 37°C (Appendix entry 4.2) (Wentworth *et al.*, 1996). *Ex vivo* studies with this abzyme and human colonic carcinoma (LoVo) cells led to a marked reduction in cell viability relative to controls. This cytotoxic activity was reproduced exactly by the Fab derived from EA11-D7 and was fully inhibited by a stoichiometric amount of the TSA [132b]. Using EA11-D7 at 0.64 μM, some 70% of cells were killed in a 1 h incubation with prodrug [129b] and the antibody transformed a net 4.18 μmol of prodrug delivering more than $2 \times IC_{50}$ of the cytotoxic agent [130]. This performance is, however, well behind that of the bacterial carboxypeptidase CPG2 used by Zeneca in their ADEPT system (Bagshawe, 1990; Blakey *et al.*, 1995), being 10^3 slower than the enzyme and 4×10^4 inferior in selectivity ratio. Nonetheless, it is the first abzyme system to show genuine medical potential and will stimulate further work in this area.

CELL VIABILITY AS AN ABZYME SCREEN

A report from Benkovic describes a new method of selecting Fabs from the whole immunological repertoire in order to facilitate a metabolic process (Smiley and Benkovic, 1994). A cDNA library for antibodies was raised against hapten [137] and then expressed in a particular strain of *E. coli* devoid of any native orotic acid decarboxylase (OCDase) activity (Fig. 44). The bacteria were then established in a pyrimidine-free medium where only those bacteria could grow which expressed an antibody capable of providing pyrimidines essential for DNA synthesis, and hence bacterial growth. Six colonies expressing an active antibody fragment were found viable in a screen of 16 000 transformants (Appendix entry 9.2). The remarkable feature of this

[5] A ρ/σ^- plot would have an even flatter slope.



KEY
PRTase - phosphoribosyl transferase
ODCase - OMP decarboxylase
OMP - orotidine 5'-phosphate
UMP - uridine 5'-phosphate

Fig. 44 Pathways for uridylate biosynthesis. Mutants lacking enzymes PRTase or ODCase can complete a route to UMP provided by an antibody orotate decarboxylase in conjunction with the naturally occurring uracil PRTase. Decarboxylation of orotic acid [135] is thought to proceed through the transition state [136], for which the hapten [137] was developed (Smiley and Benkovic, 1994).

system is that OCDase, which catalyses the decarboxylation of orotidylic acid to UMP (Fig. 44), is thought to be at the top end of performance by any enzyme in accelerating this decarboxylation by some 10^{17}-fold (Radzicka and Wolfenden, 1995).

This example of antibody catalysis illustrates the ability of abzymes to implant cell viability in the face of a damaged or deleted gene for an essential metabolic process. The medical opportunities for applications of such catalysis are clear.

There is sufficient encouragement in these examples to show that out of all the prospects for the future development of catalytic antibodies, those in the field of medicine, where selectivity in transformation of unusual substrates may be of greater importance than sheer velocity of turnover of substrate, may well rank highest.

10 Industrial potential of abzymes

In view of the tremendous interest in biocatalysis, it is not surprising that only a decade after the début of catalytic antibodies a vast literature has developed

documenting them. The field of research workers is international, with groups from three continents showing activity in the area. The potential of "designer enzymes" is already becoming a reality in relation to both the chemical industry and the pharmaceutical field.

Over 70 different chemical reactions, ranging from hydrolyses to carbon–carbon bond-forming reactions, have been catalysed by antibodies and their application to general synthetic organic chemistry seems promising. Typical Michaelis constants (K_m) lie in the range 10 μM to 1 mM and binding selectivity for the TSA over the substrate is in the range 10–10^5-fold. It therefore appears that antibodies have fulfilled expectations that they would be capable of comparable substrate discrimination to enzymes but over a wider range of substrate types than anticipated, and especially effective when programmed to a designated substrate. The range of reactions that may be catalysed by antibodies appears to be limited only by a sufficient knowledge of the transition state for any given transformation combined with synthetic accessibility to a stable TSA.

On the other hand, abzymes are generally able to accelerate reactions by at most 10^7 times the rate of the spontaneous process. It has to be said that scientists at large are looking for a major step forward in antibody catalysis to achieve rate accelerations up to 10^9 that would establish abzymes as a feature of synthetically useful biotransformations. At the same time, it is essential to demonstrate that product inhibition is not an obstacle to the scaled-up use of abzymes.

In relation to synthesis to deliver usable amounts of product, Lerner has shown that stereoselective reactions can be performed on a gram scale, as in the enantioselective hydrolysis of a 2-benzylcyclopentenyl methyl ether to the corresponding (*S*)-2-benzylcyclopentanone of high *ee* (Appendix entry 7.4A) (Reymond *et al.*, 1994). In addition, Janda has described an automated method of transposing antibody-catalysed transformations of organic molecules onto the multigram scale by employment of a biphasic system. The viability of this system was demonstrated by an epoxide ring-closing antibody, 26D9, to transform 2.2 g of substrate, corresponding to a turnover of 127 molecules per catalytic site in each batch process. This proves that the abzyme does not experience inhibition by product (Appendix entry 14.1) (Shevlin *et al.*, 1994). It would appear that an improvement in abzyme performance of little more than two orders of magnitude is needed before catalytic antibodies can be put to work in bioreactors and participate in kilogram scale production.

Lastly, two technical features of antibody production may be valuable for the future production of cheaper abzymes of commercial value. First, the use of polyclonal catalysts, primarily from sheep, has had a tough early passage but now appears to be established for a wide range to transformations (Gallacher *et al.*, 1990, 1992; Stephens and Iverson, 1993; Tubul *et al.*, 1994; Basmadjian *et al.*, 1995; Wallace and Iverson, 1996). While these catalysts may not lend themselves to detailed examination by physical organic chemistry, they have the potential to deliver catalysis at a much lower cost. Secondly, as science becomes "greener" and animal experimentation is more tightly regulated, approaches to screening antibodies with *in vitro* libraries may become a more important component of this field of work. Thomas has made a beginning with *in vitro* immunization and shown that useful catalysis can be identified (Stahl *et al.*, 1995). While there are some limitations in this system, notably the relatively low substrate affinity of antibodies generated in this way, it is capable of refinement and may become a useful component of future abzyme selection systems.

11 Conclusions

On an evolutionary time scale, abzyme research is just reaching adolescence (Thomas, 1996), yet already over 80 different antibody-catalysed chemical reactions have been catalogued during its first decade of life. The details uncovered concerning the mechanisms of abzyme-catalysed reactions have been richer than expected. The diversity of "designer" catalysts has been explored, with the potential impact on the field of medicine and the production of fine chemicals being implicated. However, the immaturity of antibody catalysis has been exposed by its inefficiency, which, in spite of intense research efforts to improve all aspects of abzyme generation, continues to hinder wide-scale acknowledgement of its contribution to biocatalysis, particularly from under the shadow of powerful enzymes.

There now exists sufficient literature about catalytic antibodies, not only in terms of their kinetic behaviour (Appendix) but also through structural information derived from X-ray crystallographic data (Golinelli-Pimpaneau *et al.*, 1994; Haynes *et al.*, 1994; Zhou *et al.*, 1994) and 3-D modelling of protein sequences (Roberts *et al.*, 1994), that it has become possible to speculate on a more general basis concerning the scope, limitations and realistic future of the field (Stewart and Benkovic, 1995; Kirby, 1996). In terms of transition state stabilization, catalytic antibodies have been shown to recognize features of the putative transition state structure encoded by their haptens with affinity constants in the nanomolar region, whereas it has been estimated that enzymes can achieve transition state complementarity with association constants of the order of 10^{-24} M to deliver rate accelerations of up to 10^{17}-fold (Radzicka and Wolfenden, 1995). The whole subject of binding energy and catalysis has been authoritatively and critically reviewed by Mader and Bartlett (1997), with especial focus on the relationship between transition state analogues and catalytic antibodies. Enzymes have evolved to interact with every species along the reaction pathways that they catalyse, whereas our manipulation of the immune system is still relatively simplistic, using a single hapten to stimulate a full, often multistep, reaction sequence of catalysis. The serendipity that may be involved in the isolation of an efficient antibody

catalyst is now well appreciated, while recent studies have shown that non-specific binding proteins such as BSA may display catalysis approaching the level of abzymes, albeit without any substrate selectivity (Hollfelder et al., 1996).

All of this serves to emphasize the fact that protein recognition of discrete high-energy reaction intermediates does not necessarily translate into efficient protein catalysis. However, the improvements in hapten design and antibody generation strategies described above are being used to highlight more intricate catalytic features. Charged and nucleophilic active-site residues (Zhong et al., 1997), substrate distortion (Datta et al., 1996; Yli-Kauhaluoma et al., 1996), desolvation and proximity effects have all now been identified as components of antibody-mediated catalysis. Using structural information available for an ever-increasing number of catalytic antibodies, manipulation of the antibody combining site is now attainable using procedures such as chemical modification (Pollack and Schultz, 1989; Schultz, 1989) and mutagenesis (Jackson et al., 1991; Stewart et al., 1994; Kast et al., 1996) to pinpoint or to improve the action of abzymes. The semi-rational design of antibody catalysts using a combination of such techniques is also supporting the systematic dissection of these primitive protein catalytic systems so as to provide valuable information concerning the origin and significance of catalytic mechanisms employed in enzymes.

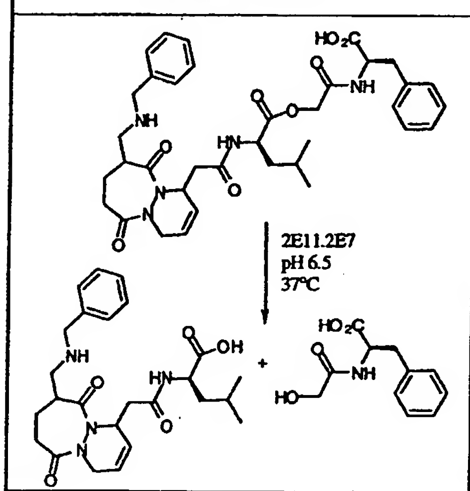
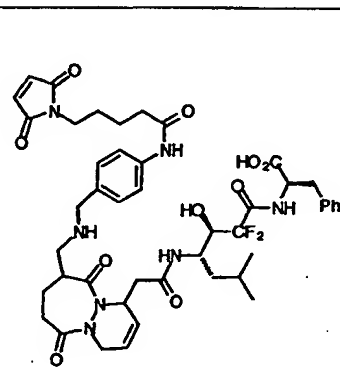
If the ultimate worth of antibody catalysts is to be more than academic, then the key must be found in their programmability. Here, the capabilities of abzymes such as their promotion of disfavoured processes and selectivity for substrates and transformations for which there are no known enzymes may offer prospects more significant than the further chasing after enzyme performance.

After all, the tortoise has a stable ecological niche!

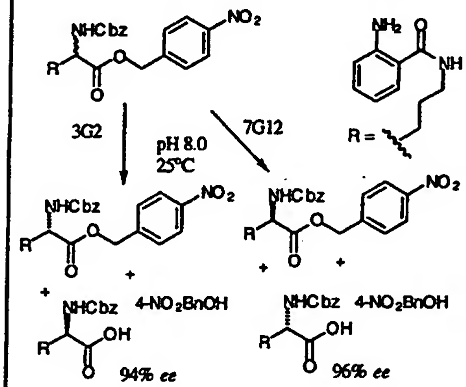
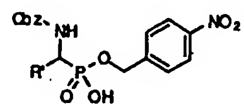
Appendix. Catalogue of antibody-catalysed processes
For key to references via entry numbers, see p. 382

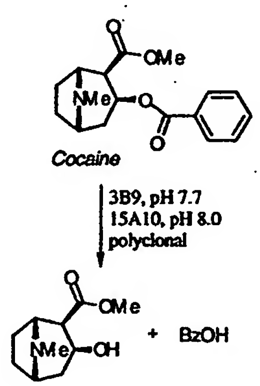
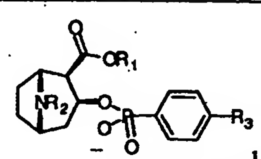
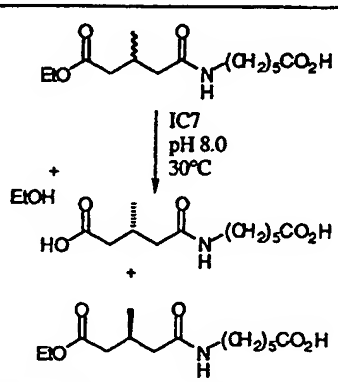
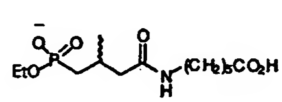
HYDROLYTIC AND DISSOCIATIVE PROCESSES

1. Aliphatic ester hydrolysis

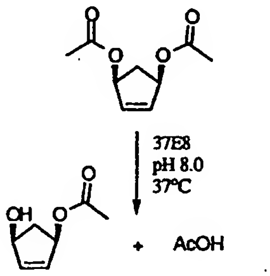
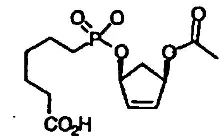
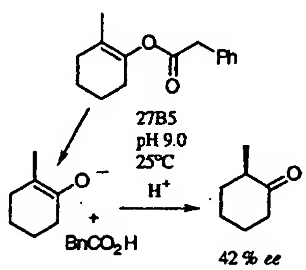
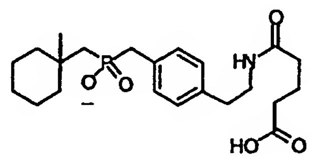
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA* $K_i < 1.0$ μM	4.4×10^3	8.0	nr	**1.1**

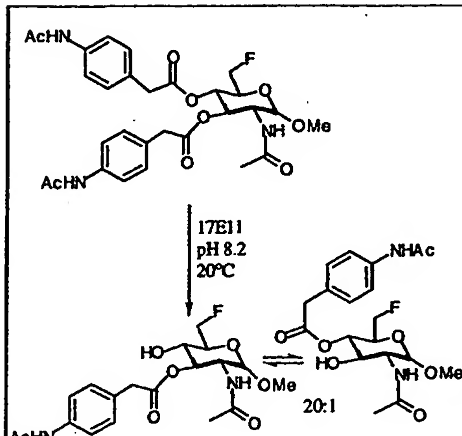
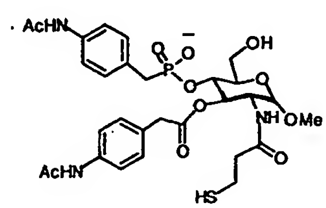
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 $R' = (CH_2)_4CO_2H$ 7G12 3G2 *TSA* 7G12: K_i 1.9×10^{-2} μM 3G2: K_i 4.7×10^{-2} μM	1.3×10^1 5.4	7.0×10^{-2} 3.3×10^{-2}	3.7×10^3 1.7×10^3	1.2

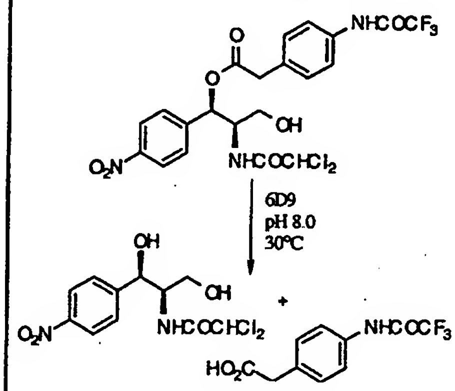
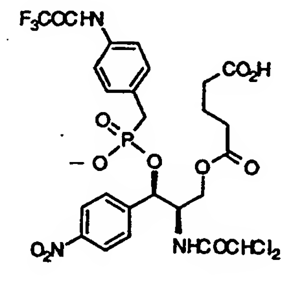
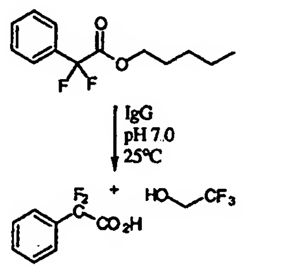
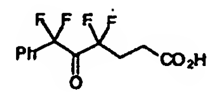
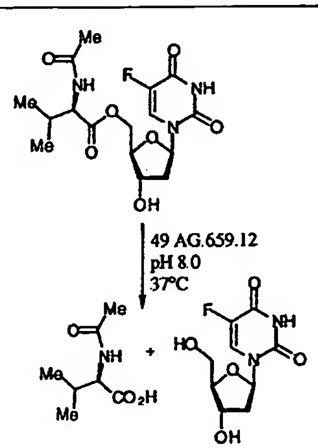
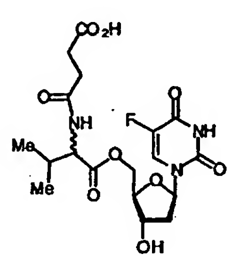
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 *Cocaine* 3B9, pH 7.7 15A10, pH 8.0 polyclonal + BzOH	 3B9 15A10 $R_1 = (CH_2)_3NHCO(CH_2)_2CO_2H$, $R_2 = Me, R_3 = H$ *TSA* 3B9: $K_i < 2.0$ μM *Vaccine immunogen* Polyclonal $R_1 = Me, R_2 = Me, R_3 = NH\text{-}DT$ $R_1 = Me, R_2 = DT, R_3 = H$ $R_1 = DT, R_2 = Me, R_3 = H$	4.9×10^2 2.2×10^2 nr	1.1×10^{-1} 2.3 nr	5.4×10^2 2.3×10^4 nr	1.3
 IC7 pH 8.0 30°C	 *TSA* K_i 4.0 μM	2.9×10^2	2.0	nr	1.4

nr, not reported.

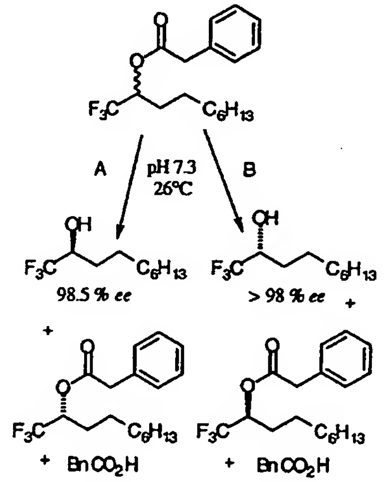
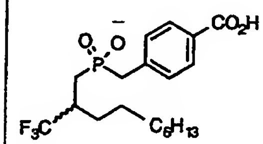
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA* K_i 7.0 μM	1.8×10^2	7.0×10^{-3}	8.8×10^1	**1.5**
	 *TSA* K_i 4.3 μM	9.9×10^2	1.0×10^{-2}	3.0×10^2	**1.6**

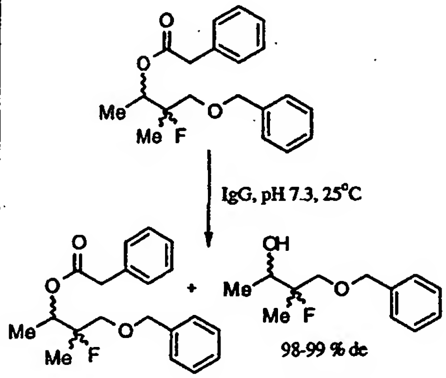
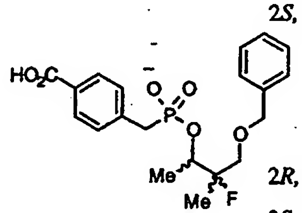
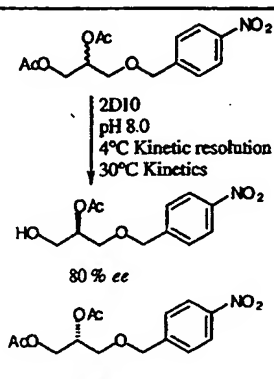
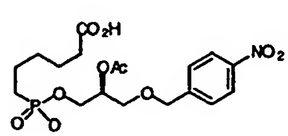
	 *TSA* K_i 2.6×10^{-2} μM	6.6	1.8×10^{-1}	2.7×10^3	**1.7**

nr, not reported.

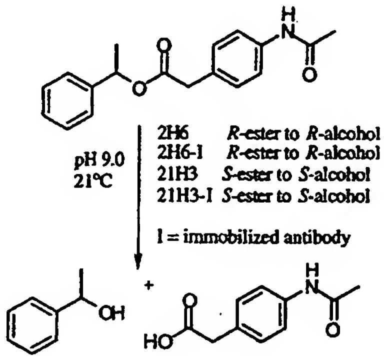
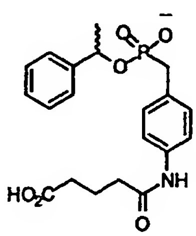
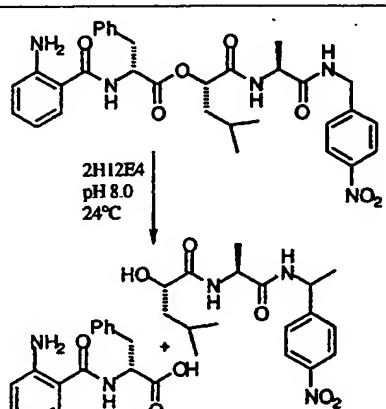
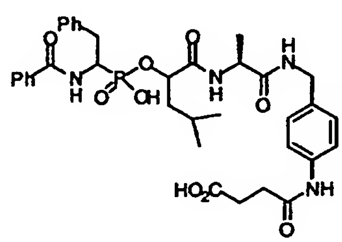
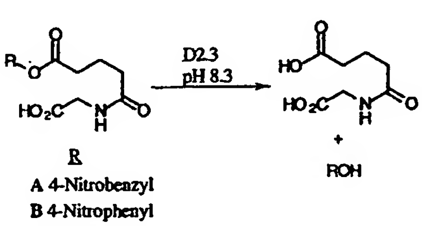
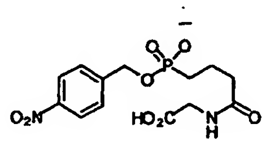
Reaction/conditions	Hapten/comments/K_i	K_m/µM	k_{cat}/min^{-1}	k_{cat}/k_{uncat}	Entry
	 *TSA* K_i 6.0×10^{-2} µM	6.4×10^{1}	1.3×10^{-1}	1.8×10^{3}	1.8
	 *TSA*	3.1×10^{2}	6.7×10^{-1}	9.1×10^{3}	1.9
	 *TSA* K_i 2.7×10^{-1} µM	2.2×10^{2}	3.0×10^{-2}	9.7×10^{2}	1.10

nr, not reported.

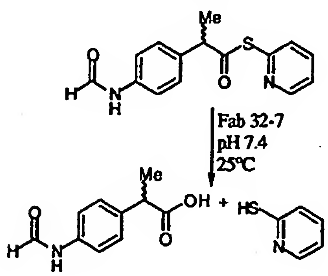
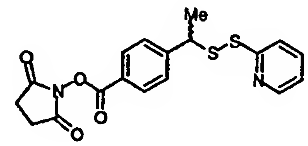
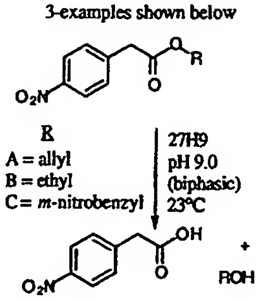
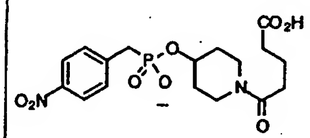
Reaction/conditions	Hapten/comments/K_i		K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 Enantiomers (+) and (−) immunized separately *TSA*	A	4.3×10^2	8.9×10^{-1}	nr	**1.11**
		B	3.9×10^2	8.6×10^{-1}	nr	

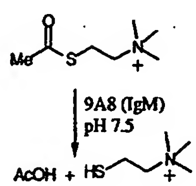
Reaction/conditions	Hapten/comments/K_i		K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA*	2*R*, 3*R*	3.9×10^2	8.8×10^{-1}	nr	**1.12**
		2*S*, 3*S*	4.0×10^2	9.1×10^{-1}	nr	
		2*R*, 3*S*	4.1×10^2	9.4×10^{-1}	nr	
		2*S*, 3*R*	3.8×10^2	8.6×10^{-1}	nr	
	 *TSA* K_i 2.8 μM		1.3×10^3	2.0	2.4×10^2	**1.13**

nr, not reported.

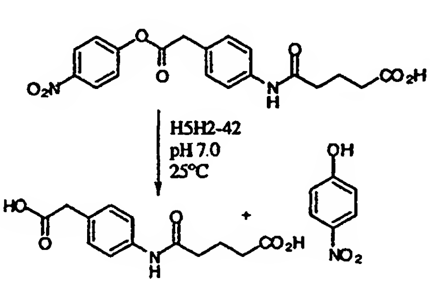
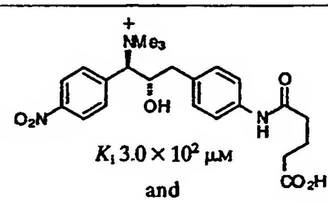
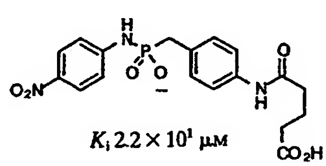
Reaction/conditions	Hapten/comments/K_i		K_m/ µM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA* 2H6: K_i 2.0 µM 21H3: K_i 1.9×10^{-1} µM	2H6	4.0×10^3	4.6	8.3×10^4	1.14
		2H6-I	2.2×10^3	4.0	7.2×10^4	
		21H3	3.9×10^2	9.0×10^{-2}	1.6×10^3	
		21H3-I	2.0×10^2	6.0×10^{-2}	1.1×10^3	
	 *TSA* K_i 2.4 µM		1.5×10^1	1.9×10^{-2}	2.7×10^2	1.15
	 *TSA*	A	2.8×10^2	7.4	2.6×10^5	1.16
		B	3.3×10^1	3.4	7.2×10^3	

nr, not reported.

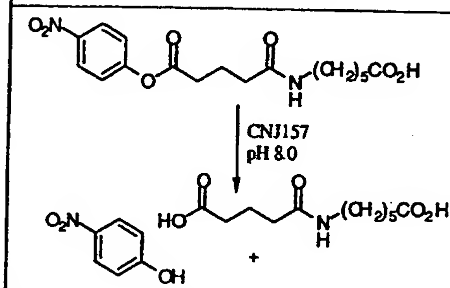
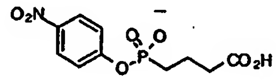
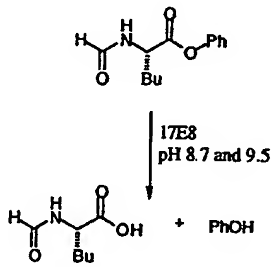
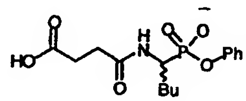
Reaction/conditions	Hapten/comments/K_i	K_m/ µM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 Fab 32-7, pH 7.4, 25°C	 in vitro *Chemical Selection*	1.0×10^2	3.0×10^{-2}	3.0×10^1	1.17
Esterase with broad substrate tolerance 3-examples shown below  R: A = allyl, B = ethyl, C = *m*-nitrobenzyl; 27H9, pH 9.0 (biphasic), 23°C	 *TSA* $K_{i\,(Substrate\,A)}$ 1.2×10^1 µM	A 3.4×10^3 B 2.8×10^3 C 1.5×10^3	6.2×10^{-2} 1.1×10^{-2} 4.7×10^{-1}	2.9×10^4 8.8×10^3 1.4×10^6	1.18

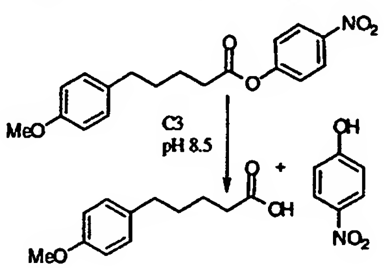
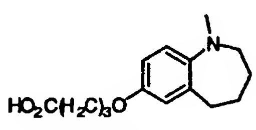
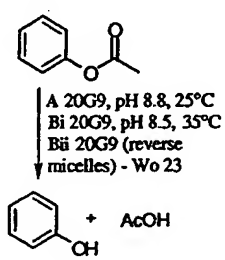
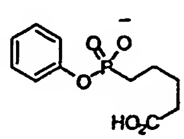
 9A8 (IgM), pH 7.5	ANTI-IDIOTYPIC CATALYSTS Antibodies elicited against mAbE-2 an anti-acetylcholinesterase antibody	6.0×10^2	4.9×10^3	4.2×10^8	1.19

2. Aryl ester hydrolysis

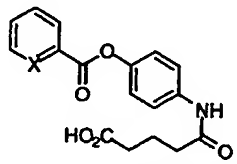
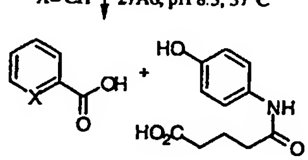
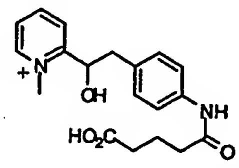
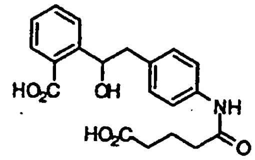
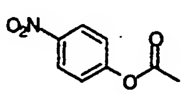
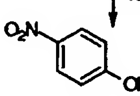
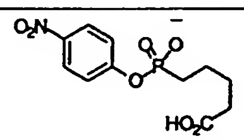
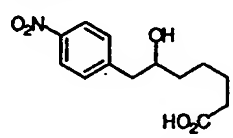
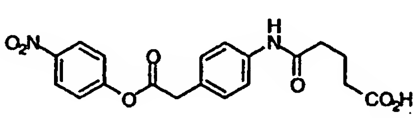
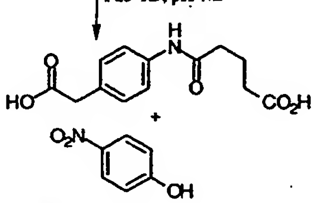
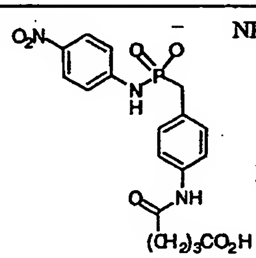
 H5H2-42, pH 7.0, 25°C	 K_i 3.0×10^2 µM and  K_i 2.2×10^1 µM *Heterologous Immunization*	2.4×10^2	1.3×10^1	6.8×10^4	2.1

nr, not reported.

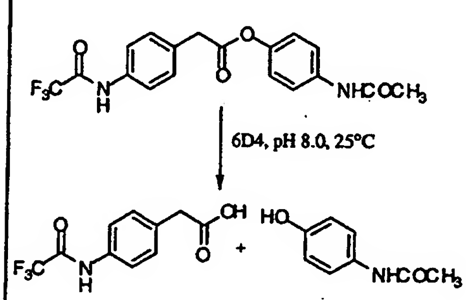
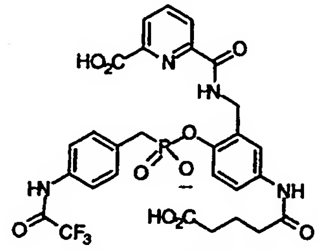
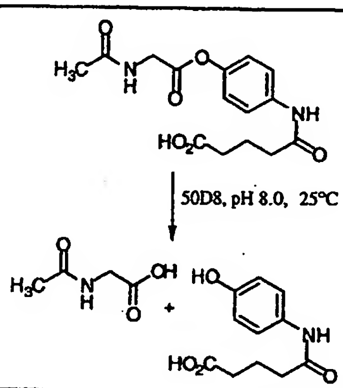
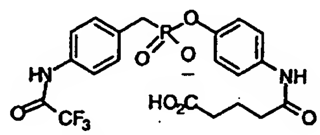
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA* K_i 3.4×10^3 μM	1.1×10^2	2.4	9.7×10^3	2.2
	 pH 8.7 pH 9.5 *TSA* K_i 5.0×10^{-1} μM	2.6×10^2 nr little variance with pH	1.0×10^2 2.2×10^2	1.3×10^4 2.2×10^4	2.3

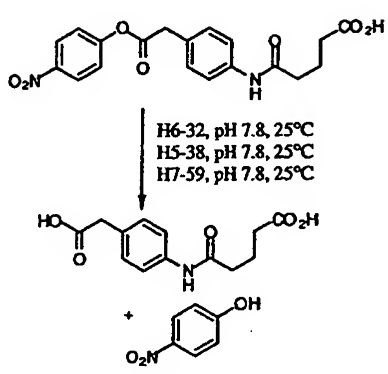
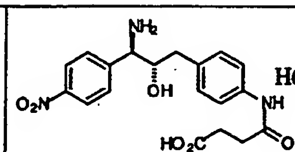
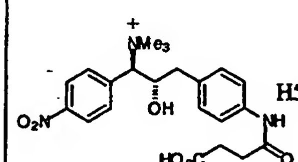
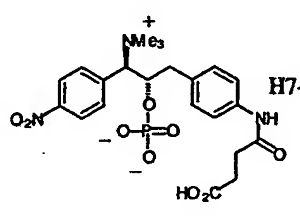
	 *TSA* K_d 2.4×10^{-2} μM	4.0×10^2	1.4×10^2	3.5×10^6	2.4
	 A Bi Bii *TSA* A: K_i 2.2×10^{-3} μM Bi: K_i 3.9×10^{-2} μM	3.6×10^1 1.6×10^2 5.7×10^2	5.4×10^1 1.9×10^1 3.9	6.9×10^1 1.7×10^4 nr	2.5

nr, not reported.

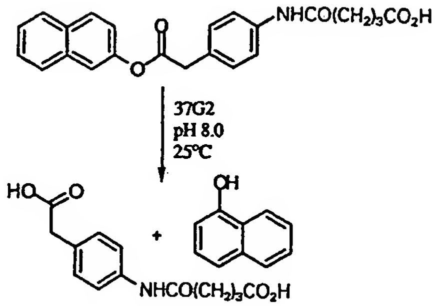
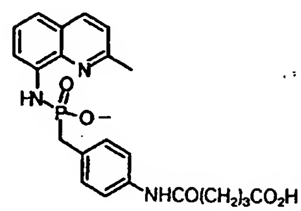
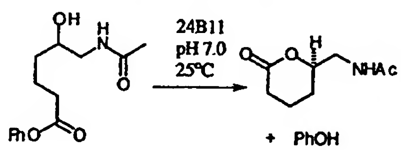
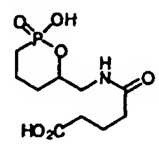
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 X = CH 30C6, pH 7.2, 37°C; X = N 84A3, pH 7.0, 25°C; X = CH 27A6, pH 8.3, 37°C 	 30C6	1.1×10^3	5.0×10^{-3} (app.)	1.0×10^6	**2.6**
	84A3 (zinc dependent)	3.5	2.7	1.2×10^3	
	30C6: K_i 8.3×10^1 μM				
	 27A6	2.4×10^2	2.0×10^{-3} (app.)	nr	
	27A6: K_i 6.0 μM				
	Bait and Switch (BS)				
 KD2-260, pH 6.0, 20°C; 7K16.2, pH 7.5, 30°C  + AcOH	 KD2-260	4.9	2.5	3.1×10^3	**2.7**
	KD2-260: $K_{i(30°C)}$ 1.2×10^{-1} μM				
	 7K16.2	3.7×10^3	7.2×10^{-1}	2.3×10^3	
	7K16.2: K_i 1.4×10^2 μM				
	TSA				
 NPN43C9, pH 9.3, 25°C; Fab-1D, pH 7.2 	 NPN43C9	5.3×10^1	1.5×10^3 (estimate based on pH rate profile)	2.7×10^4	**2.8**
	Fab-1D	1.1×10^2	2.5×10^1	nr	
	TSA				
	NPN43C9: $K_{d\ (pH7)}$ 1.0 μM				

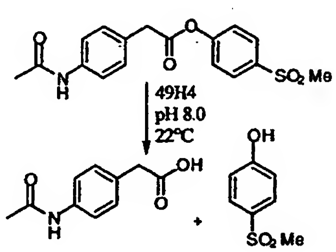
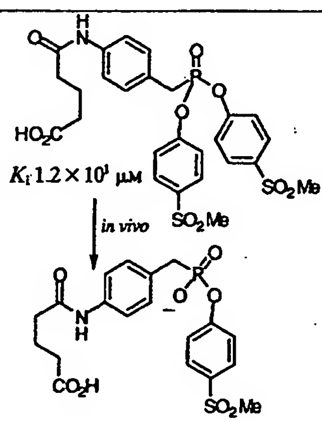
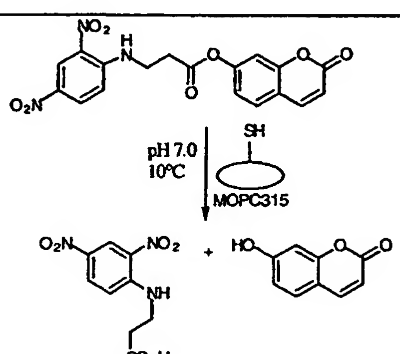
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 6D4: K_i 1.6×10^{-1} μM *TSA*	1.9	1.6	9.6×10^2	2.9
	 50D8: K_i 5.0×10^{-2} μM *TSA*	1.5×10^3	1.2×10^3	6.3×10^6	2.10

	 H6-32 H6-32: K_i 3.6×10^2 μM	8.5×10^2	7.1×10^{-1}	2.4×10^3	2.11
	 H5-38 H5-38: K_i 5.0 μM	8.7×10^2	1.0	3.3×10^3	
	 H7-59 H7-59: K_i 2.3×10^2 μM	1.1×10^3	4.9×10^{-1}	1.6×10^3	

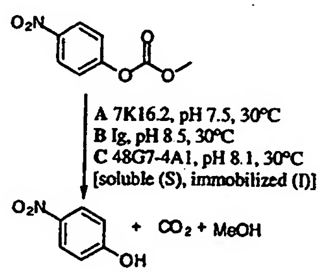
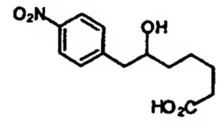
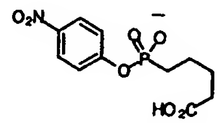
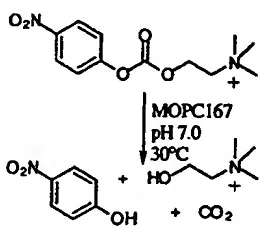
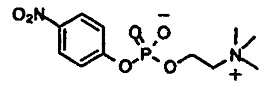
nr, not reported.

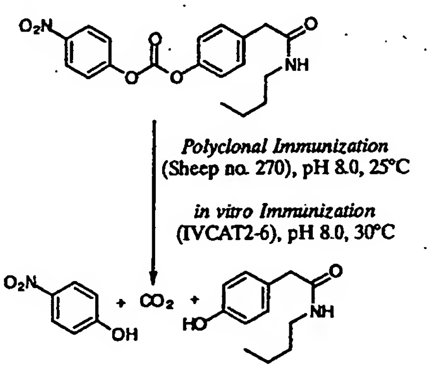
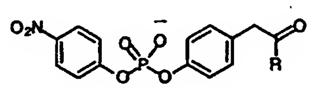
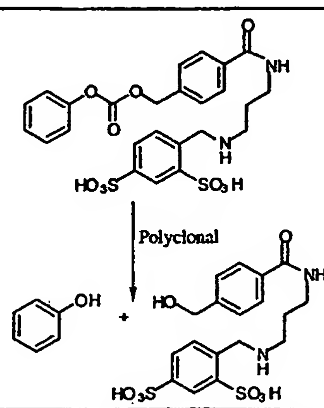
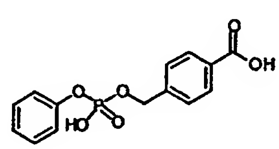
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA* K_i 5.0×10^{-1} μM	7.7×10^2	2.0×10^{-1}	2.0×10^3	2.12
Lactone formation 	 *TSA* K_i 2.5×10^{-1} μM	7.6×10^1	5.0×10^{-1}	1.7×10^2	2.13

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 K_i 1.2×10^1 μM *Reactive Immunization (RI)*	3.0×10^2	3.1×10^1	6.7×10^3	2.14
	*Semisynthetic antibodies* Nucleophilic thiol groups were introduced into a 2,4-dinitrophenyl ligand specific antibody binding site by chemical modification K_i (DNP-Gly) 2.5×10^{-1} μM	1.2	8.7×10^{-1}	6.0×10^4	2.15

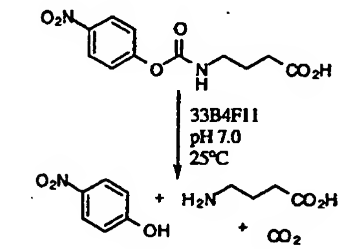
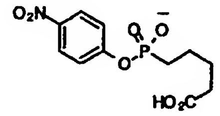
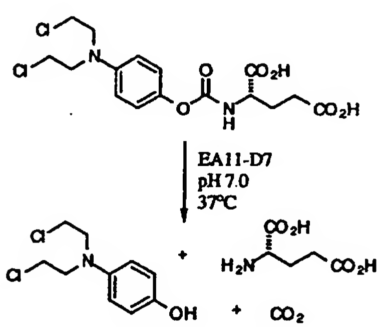
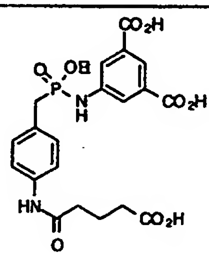
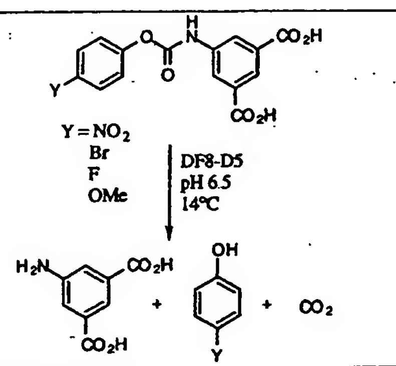
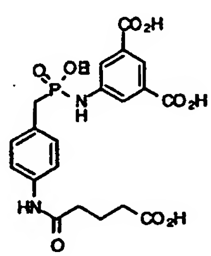
nr, not reported

3. *Carbonate hydrolysis*

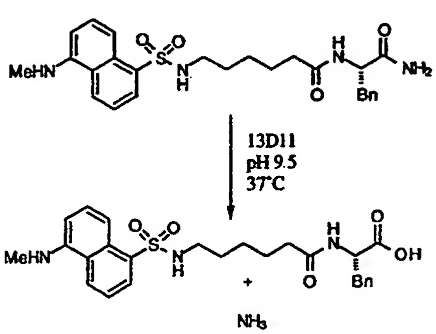
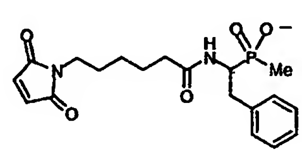
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 A 7K16.2, pH 7.5, 30°C B Ig, pH 8.5, 30°C C 48G7-4A1, pH 8.1, 30°C [soluble (S), immobilized (I)]	 A	3.3×10^3	3.1×10^{-1}	9.3×10^2	3.1
	 B	6.6×10^2	1.4	8.1×10^2	
	C(S)	4.3×10^2	4.0×10^1	2.3×10^4	
	C(I)	6.8×10^2	2.3×10^1	1.3×10^4	
	B: K_i 3.3 μM *TSA*				
 MOPC167 pH 7.0 30°C	 *TSA* K_i 5.0 μM	2.1×10^2	4.0×10^{-1}	7.7×10^2	3.2

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 *Polyclonal Immunization* (Sheep no. 270), pH 8.0, 25°C *in vitro Imminization* (IVCAT2-6), pH 8.0, 30°C	Sheep no. 270	3.3	1.7×10^2 (based on 1% active protein)	1.5×10^4	3.3
	 IVCAT2-6	9.8×10^2	7.2×10^1	5.5×10^3	
	Sheep no. 270, R = OH IVCAT2-6, R = $NH(CH_2)_3CH_3$ *TSA* Sheep no. 270: K_i 9.0×10^{-3} μM IVCAT2-6: K_i 2.0×10^2 μM				
 Polyclonal	 *TSA* $K_{d\,(app)}$ 6.9 μM	8.9×10^1 (app.)	2.1×10^{-1} (app.)	4.3×10^3 (app.)	3.4

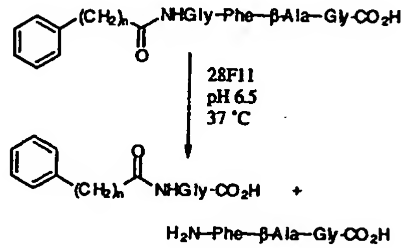
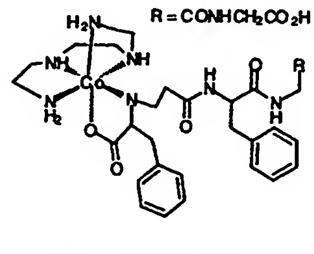
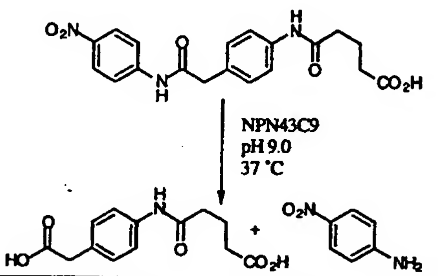
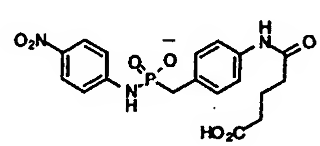
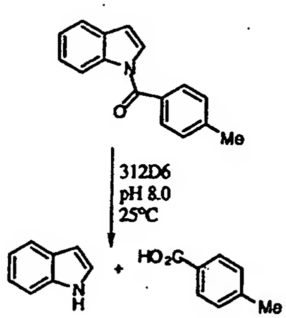
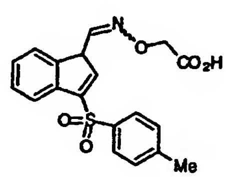
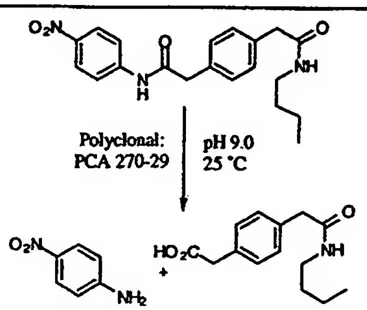
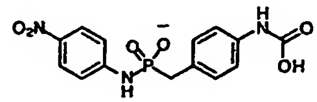
4. Carbamate ester hydrolysis

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA* K_i 1.0 × 10^{-1} μM	5.5	1.5	2.6 × 10^2	4.1
	 *TSA* K_d 2.0 × 10^8 M	2.0 × 10^2	1.9	nr	4.2
	 *TSA* Y: NO_2	1.2 × 10^2	1.8 × 10^1	3.0 × 10^2	4.3
	Br	8.0 × 10^1	6.0	1.0 × 10^4	
	F	4.1 × 10^1	7.2	4.0 × 10^4	
	MeO	5.8 × 10^1	4.9	1.2 × 10^6	

5. Amide hydrolysis

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA* K_i 1.4 × 10^1 μM	4.3 × 10^2	9.9 × 10^{-6}	1.3 × 10^2	5.1

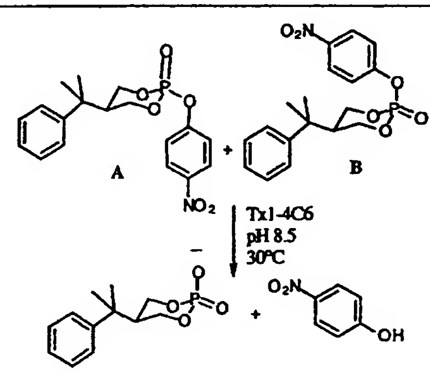
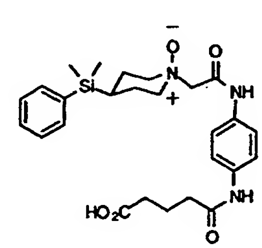
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 28F11, pH 6.5, 37 °C	 *Metal complex cofactor*	nr	3.6×10^{-2}	2×10^5	5.2
 NPN43C9, pH 9.0, 37 °C	 *TSA* K_i 1.0×10^1 μM	5.6×10^2	8.0×10^{-2}	2.5×10^5	5.3
 312D6, pH 8.0, 25°C	 *TSA*	3.6×10^1	4.5×10^{-4}	7.5×10^2	5.4
 Polyclonal: PCA 270-29, pH 9.0, 25 °C	 *TSA*	5.4	3.6×10^{-1} (based on 1% active protein)	1.1×10^3	5.5

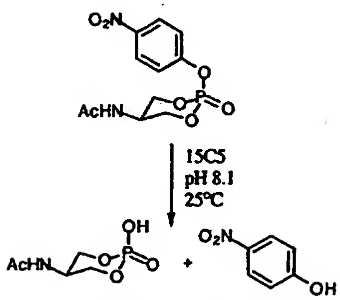
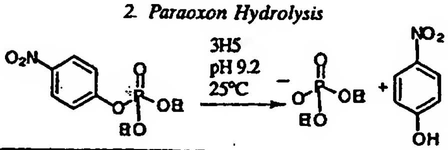
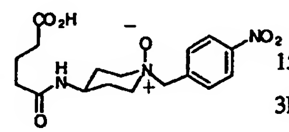
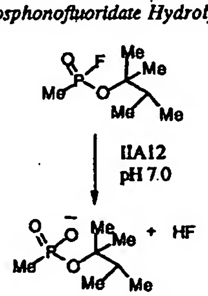
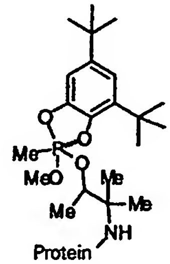
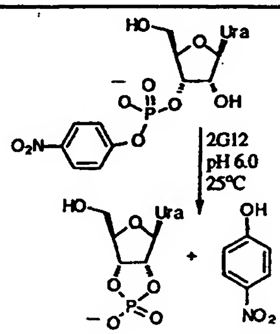
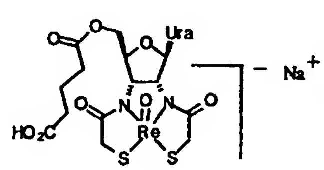
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Gln^{16} —/— Met^{17} VIP $\xrightarrow{\text{Fab, pH 8.5, 38 °C}}$ VIP (1-16) + VIP (17-28)	*Autoantibodies* Human serum IgG fraction was found to hydrolyse vasoactive intestinal polypeptide (VIP). Unknown immunogen	3.8×10^{-2}	1.6×10^{1}	nr	5.6
Tg $\xrightarrow{\text{Polyclonal Tg-Ab}}$ Tg-fragments	*Autoantibodies* Human serum IgG fraction was found to hydrolyse thyroglobulin (Tg). Unknown immunogen	3.9×10^{-2}	3.9×10^{-3}	nr	5.7
Boc-EAR-MCA $\xrightarrow{\text{BJP-B6}}$ Boc-EAR + MCA	*Autoantibodies* Bence Jones proteins (BJPs) (monoclonal antibody light chains) isolated from the urine of multiple myeloma patients, were found to hydrolyse peptide methylcoumarin amide peptide-MCA substrates	1.5×10^{1}	3.3×10^{-2}	nr	5.8

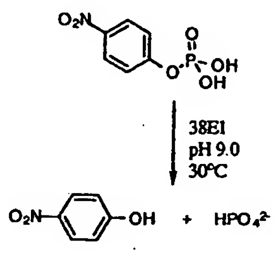
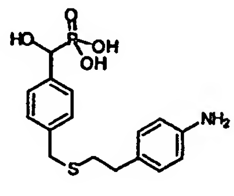
6. Phosphate ester hydrolysis

Reaction/conditions	Hapten/comments/K_i		K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *Electrostatic TS Stabilization* $K_{d\,(\text{fluorescence quench})}$ 6.7×10^{-1} μM	A	1.8×10^{1}	1.9×10^{-3}	3.6×10^{2}	6.1
		B	3.6×10^{1}	1.0×10^{-2}	9.8×10^{2}	

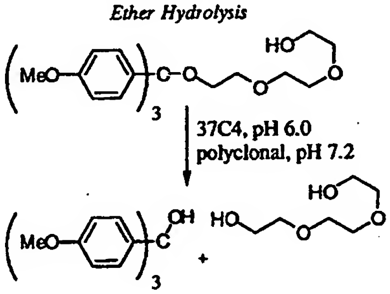
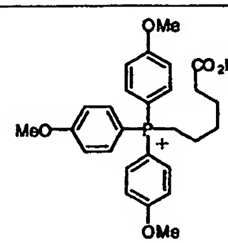
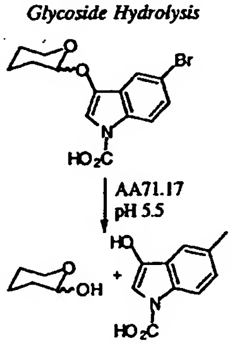
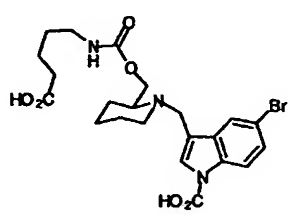
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
1. N-Acyl Serinol Triester Hydrolysis  *2. Paraoxon Hydrolysis* 	 15C5 3H5 *Electrostatic TS Stabilisation and BS* 3H5: $K_{i(pH\,9.3)}$ 9.8×10^{-1} μM	8.7×10^{1} 5.1×10^{3}	2.7×10^{-3} 2.0×10^{-3}	1.3×10^{2} 3.5×10^{2}	6.2
Phosphonofluoridate Hydrolysis 	 *TSA*	3.3×10^{2}	4.0	5.5×10^{3}	6.3
	 *TSA* $K_i < 4.0 \times 10^{-1}$ μM	2.4×10^{2}	9.2×10^{-2}	3.1×10^{2}	6.4

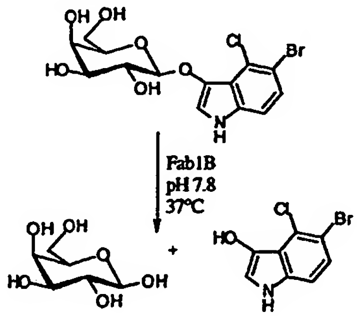
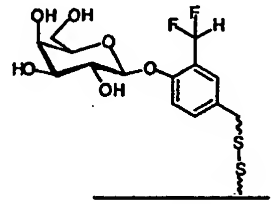
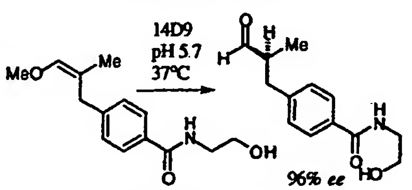
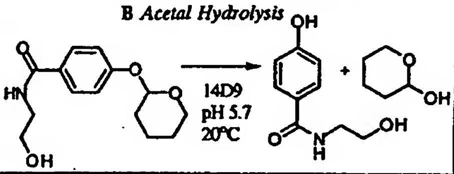
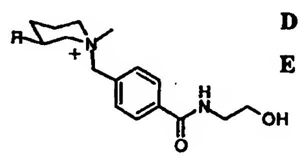
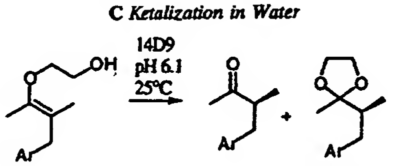
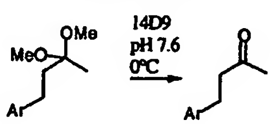
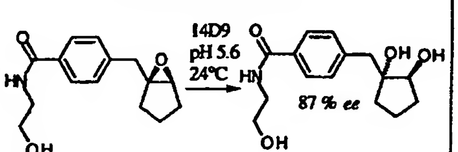
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Plasmid DNA (pUC18) → Nicked DNA Fab fragment from an IgG purified from human sera pH 7.5, 30°C	*Autoantibodies* Human serum IgG fraction (Fab) was found to hydrolyse DNA. Unknown immunogen	4.3×10^1	1.4×10^1	nr	6.5
	 K_i 3.4×10^1 μM	1.6×10^2	1.2×10^{-3}	8.0×10^3	6.6

7. Miscellaneous hydrolyses

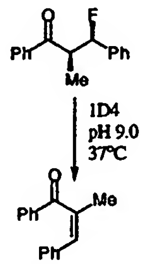
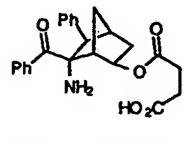
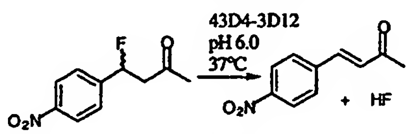
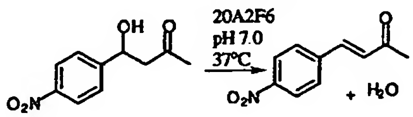
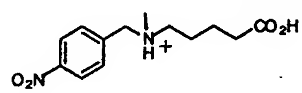
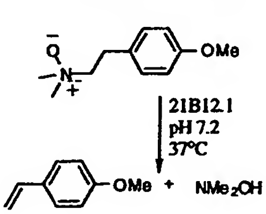
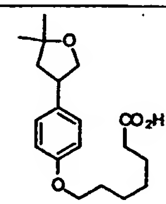
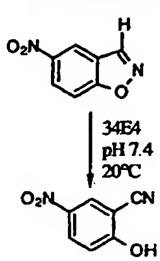
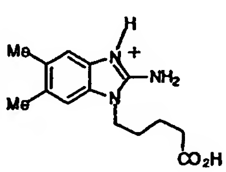
Ether Hydrolysis 	 37C4 polyclonal *TSA* 37C4: K_d 2.5×10^{-2} μM	3.1×10^1 3.1×10^1	1.0×10^{-1} 2.0×10^{-2} (based on 12% active protein)	2.7×10^2 1.3×10^2	7.1
Glycoside Hydrolysis 	 *TSA/BS* K_i 3.5×10^1 μM	3.2×10^2	1.5×10^{-2}	nr	7.2

nr, not reported.

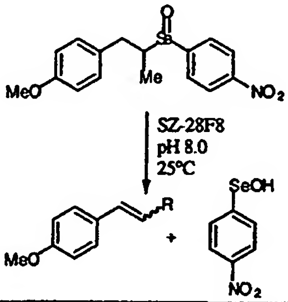
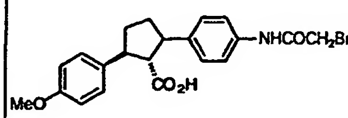
Reaction/conditions	Hapten/comments/K_i	K_m/ µM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 in vitro *Chemical Selection* K_i 1.5×10^1 µM	5.3×10^2	7.0×10^{-3}	7.0×10^4	7.3
A *Enol Ether Hydrolysis*  B *Acetal Hydrolysis* 					7.4
	A	3.4×10^2	5.7×10^{-3}	2.5×10^3	
	B	1.0×10^2	4.7×10^{-3}	7.0×10^1	
	C	5.0×10^1	7.2×10^{-2}	6.0×10^2	
	D	2.3×10^2	1.0×10^{-2}	4.3×10^2	
	E	2.5×10^1	1.5×10^{-3}	4.4×10^2	
C *Ketalization in Water*  12 %, >99 % *ee* D *Ketal Hydrolysis*  E *Epoxide Hydrolysis* 	R = $CH_2NHCO(CH_2)_3CO_2H$ *BS* A: K_d 1.0×10^{-2} µM				7.4

nr, not reported.

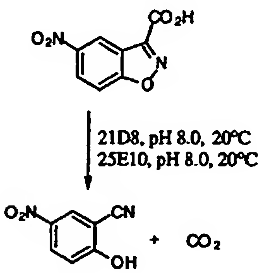
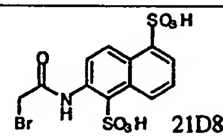
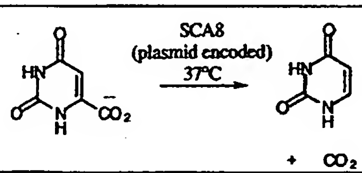
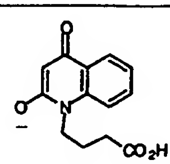
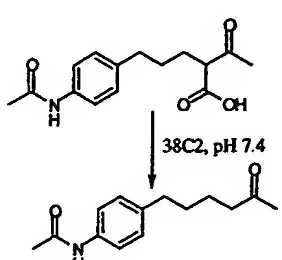
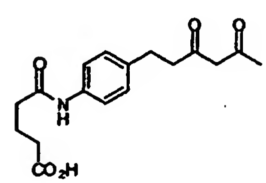
8. Eliminations

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Disfavoured syn Elimination 	 *BS and Entropic Trap*	2.1×10^2	3.0×10^{-3}	nr	8.1
1. HF Elimination  *2. Dehydration* 	43D4-3D21 20A2F6  *BS* 43D4-3D12: K_i 2.9×10^{-1} μM 20A2F6: K_i 1.6×10^1 μM	1.8×10^2 1.1×10^3	1.9×10^{-1} 3.5×10^{-4}	8.8×10^4 1.2×10^3	8.2
2,3-Cope Elimination 	 *TSA* K_i 2.0×10^{-1} μM	2.4×10^2	2.4×10^{-5}	9.1×10^2	8.3
E2 Elimination 	 *BS*	1.2×10^2	4.0×10^1	2.1×10^4	8.4

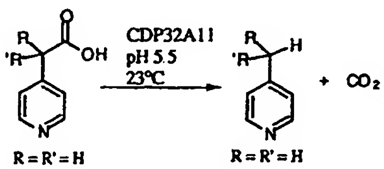
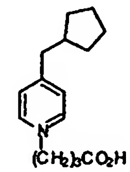
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Selenoxide Elimination 	 *TSA* K_i 8.2×10^{-2} μM	1.5	1.8×10^{-1}	1.6×10^{2}	8.5

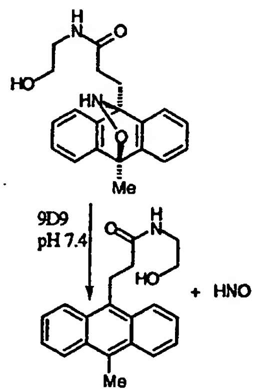
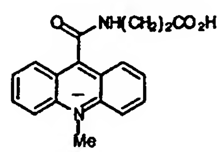
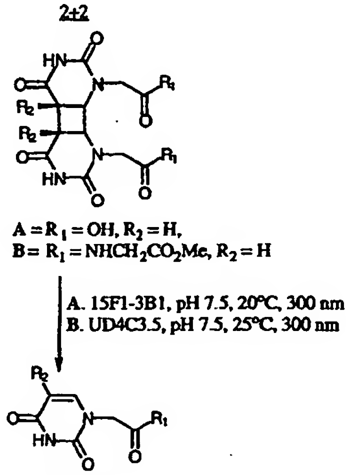
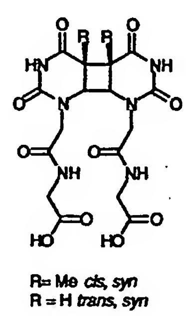
9. Decarboxylations

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 21D8	1.7×10^{2}	1.7×10^{1}	1.9×10^{4}	9.1
	25E10 *Medium Effect* 21D8: K_i 6.8×10^{-3} μM 25E10: K_i 2.4×10^{-3} μM	2.6×10^{2}	2.3×10^{1}	2.3×10^{4}	
		nr	2.7×10^{-4}	1.0×10^{8}	9.2
	 *Reactive Immunization*	9.5×10^{2}	1.6×10^{-1}	1.5×10^{4}	9.3

nr, not reported.

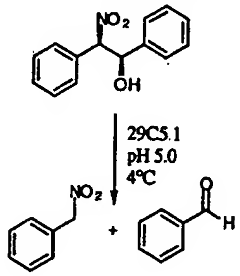
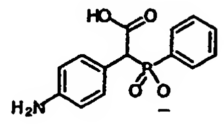
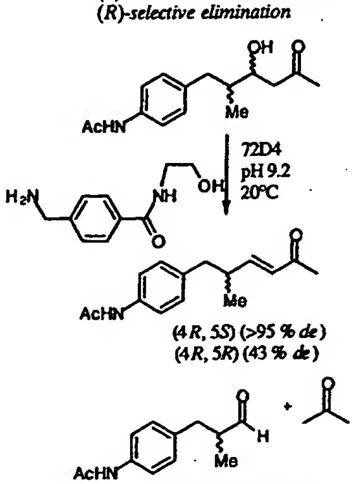
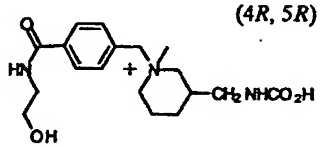
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *Medium Effect* K_i 1.0 × 10^{-2} μM	1.4 × 10^5	2.8 × 10^{-2}	1.9 × 10^5	9.4

10. Cycloreversions

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Retro Diels–Alder Reaction 	 *Heterologous Immunization* (with hydroxylated form) $K_{i\,(pH\,9.0)}$ 9.0 × 10^{-1} μM	1.3 × 10^2	7.3 × 10^{-2}	2.3 × 10^2	10.1
2+2 	 *BS* 15F1-B1: K_i < 1.0 μM UD4C3.5: K_d (fluorescence quench) 5.4 × 10^{-2} μM	A 6.5 B 2.8 × 10^2	A ~1.2 B 4.7 × 10^{-1}	A 2.2 × 10^2 B 3.8 × 10^2	10.2

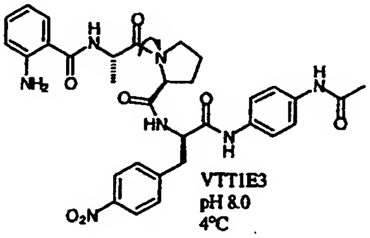
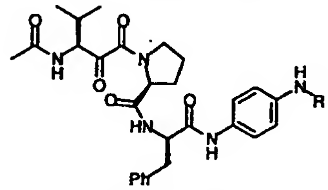
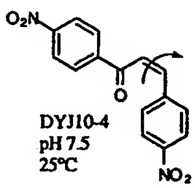
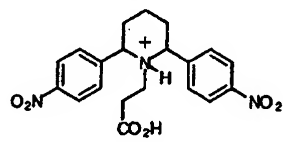
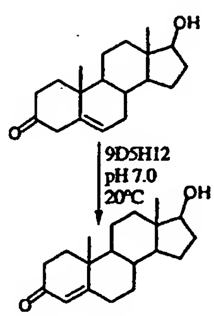
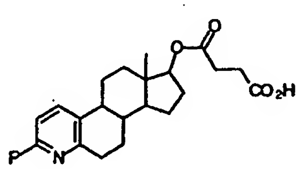
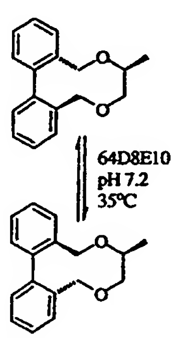
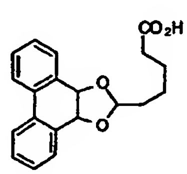
nr, not reported.

11. Retro aldol reactions

Reaction/conditions	Hapten/comments/K_i	K_m/ µM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Retro-Aldol Condensation  	 *TSA* K_i (app) 2.6 µM	k_{cat}/K_m $1.3 \times 10^2\ M^{-1}\ min^{-1}$ (k_{cat} and K_m not measured separately)		k_{imid} $2.5 \times 10^{-4}\ M^{-1}\ min^{-1}$	11.1
(*S*)-*Selective Retroaldol and* (*R*)-*selective elimination*  	(*R*)-*Selective elimination* (0.8 mM amine) (4*R*, 5*S*) (4*R*, 5*R*)  (*S*)-*Selective retroaldol* (0.8 mM amine) (4*S*, 5*S*) (4*S*, 5*R*)	$k_{cat}/K_m\ M^{-1}\ min^{-1}$ (app) 1.1×10^{-1} 2.2×10^{-2} 7.8×10^{-2} 2.2×10^{-1}		nr nr	11.2

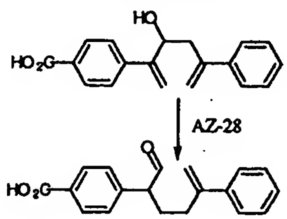
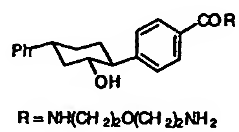
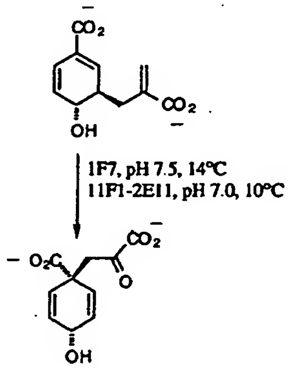
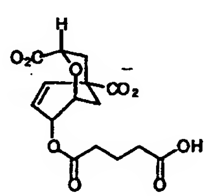
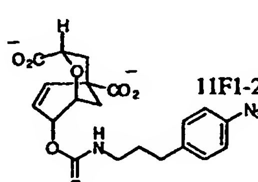
nr, not reported.

12. Isomerizations

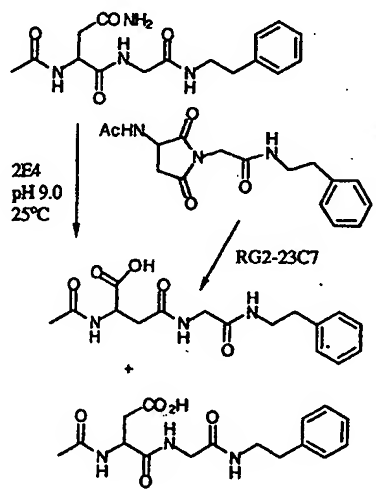
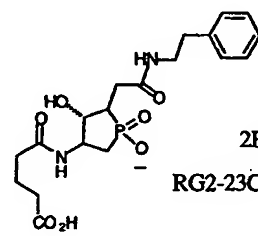
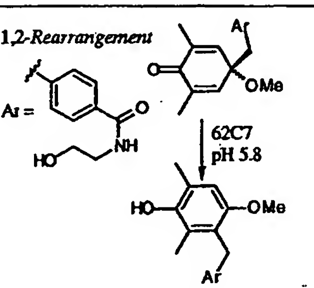
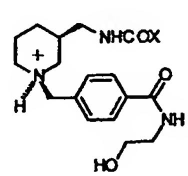
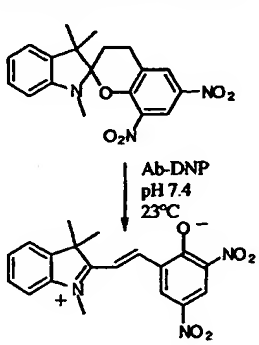
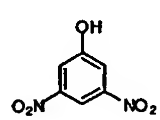
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Peptidyl-prolyl cis/trans isomerization  VTT1E3 pH 8.0 4°C	 R = CO(CH$_2$)$_3$COOH *TSA* K_i 1.0 × 10^1 μM	1.0 × 10^2	6.6	2.7 × 10^1	12.1
cis–trans *Isomerization*  DYJ10-4 pH 7.5 25°C	 *BS* K_i 6.7 μM	2.2 × 10^2	4.8	1.5 × 10^4	12.2
 9D5H12 pH 7.0 20°C	 *TSA* K_i 1.3 × 10^2 μM	1.6 × 10^2	2.3	8.3 × 10^2	12.3
 64D8E10 pH 7.2 35°C	 *TSA* K_d (BIAcore) 2.1 10^{-1} μM	4.2 × 10^2	2.6 × 10^{-3}	2.9 × 10^3	12.4

nr, not reported.

13. Rearrangements

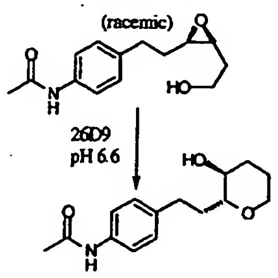
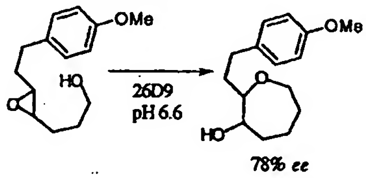
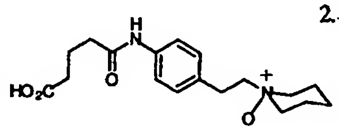
Reaction/conditions	Hapten/comments/K_i	K_m/ µM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Cope Rearrangement  AZ-28	 R = NH(CH$_2$)$_2$O(CH$_2$)$_2$NH$_2$ *TSA* K_i 3.0×10^{-2} to 1.6×10^{-1} µM	9.7×10^1 (app.) 4.9×10^1 (cor.)	2.6×10^{-2}	5.3×10^3	13.1
Claisen Rearrangement  1F7, pH 7.5, 14°C 11F1-2E11, pH 7.0, 10°C	 1F7 1F7: K_i 6.0×10^{-1} µM	4.9×10^1	2.3×10^{-2}	2.5×10^2	13.2
	 11F1-2E11 11F1-2E11: K_i 9.0 µM	2.6×10^2	2.7	1.0×10^4	

nr, not reported.

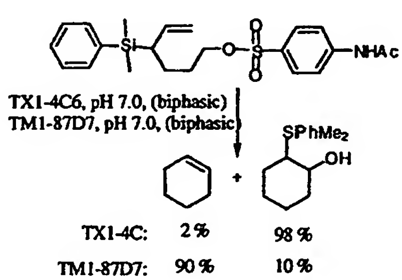
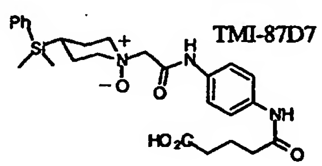
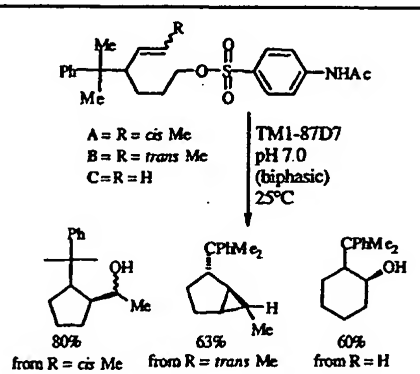
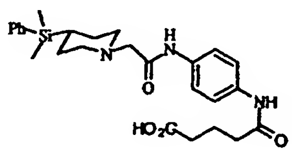
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Peptide Bond Rearrangement and Succinimide Hydrolysis 	 2E4 RG2-23C7 *TSA* 2B4: K_i 1.0×10^{-1} μM	1.9×10^2 8.3×10^{-1}	7.2×10^{-3} 3.6×10^1	7.0×10^1 nr	13.3
1,2-Rearrangement 	 *TSA*	6.7×10^2	7.3×10^{-5}	8.0×10^1	13.4
Ring-Opening of a Dinitrospyran 		1.7×10^5	1.8×10^1	1.9×10^4	13.5

nr, not reported.

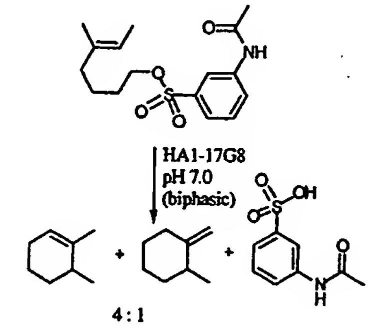
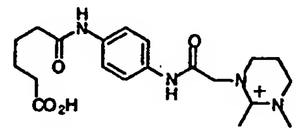
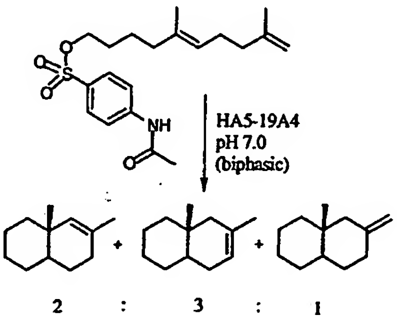
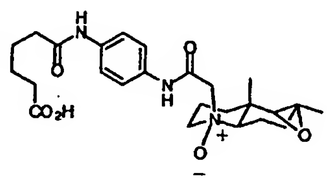
14. Epoxide opening

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
1. anti-Baldwin Ring Closure  *2. Oxepane Synthesis* 	 *TSA*	1. 3.6×10^2 2. 2.0×10^2	9.0×10^{-1} 9.0×10^{-1}	nr nr	14.1

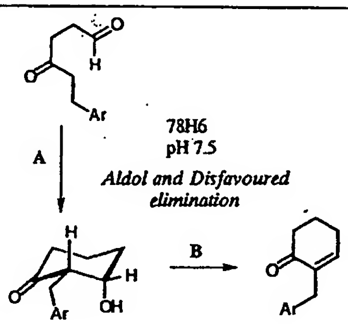
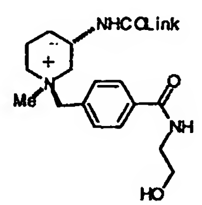
15. Cationic cyclization

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	TX1-4C6 TMI-87D7  *TSA* TX1-4C6: K_i 1.0 μM TM1-87D7: K_i 1.4 μM	2.3×10^2 2.5×10^1	2.0×10^{-2} 2.0×10^{-2}	nr nr	15.1
	 A B C *TSA* A: K_i 1.0 μM B: K_i 1.0 μM C: K_i 1.0 μM	5.8×10^1 1.0×10^2 3.1×10^1	1.3×10^{-2} 2.1×10^{-2} 1.0×10^{-2}	nr nr nr	15.2

nr, not reported.

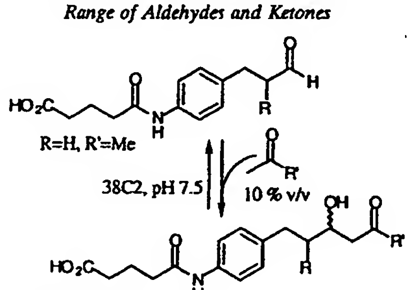
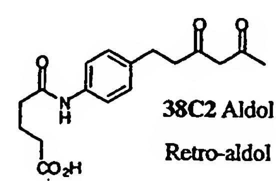
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 *TSA* IC_{50} 1.2 μM	3.5×10^1	2.5×10^{-2}	7.0×10^1	**15.3**
	 *TSA* K_i 1.4 μM	3.2×10^2	2.1×10^{-2}	2.3×10^3	**15.4**

16. Aldol reactions

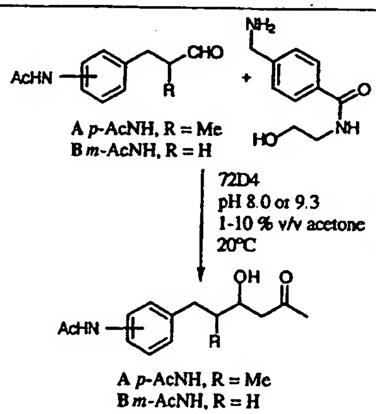
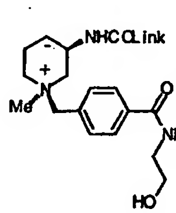
	 *BS*	A B	3.6×10^2 4.7×10^2	1.4×10^{-4} 4.9×10^{-4}	2.0×10^5 3.6×10^4	**16.1**

BIMOLECULAR ASSOCIATIVE AND SUBSTITUTION PROCESSES

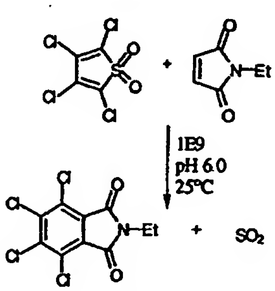
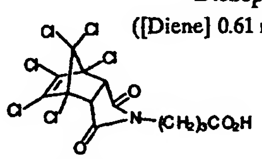
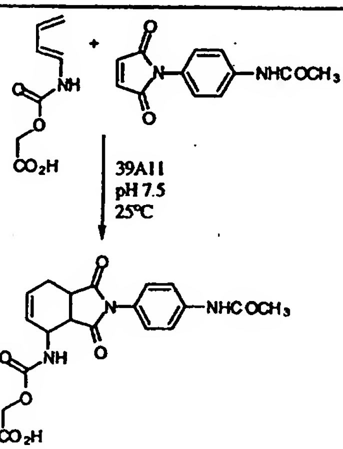
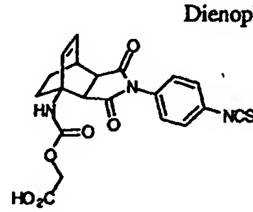
16. Aldol reactions continued

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Aldol and Retroaldol Reaction with a Range of Aldehydes and Ketones 	 **38C2 Aldol** Retro-aldol *Reactive Immunization*	1.7×10^1 5.4×10^1	6.7×10^{-3} 4.4×10^{-3}	2.9×10^4 nr	**16.2**

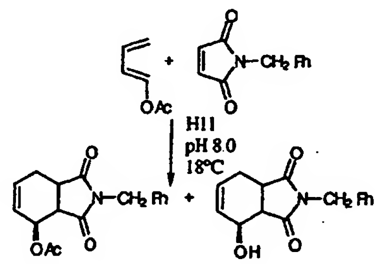
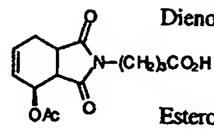
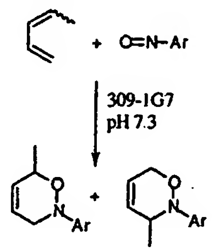
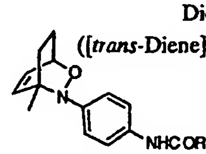
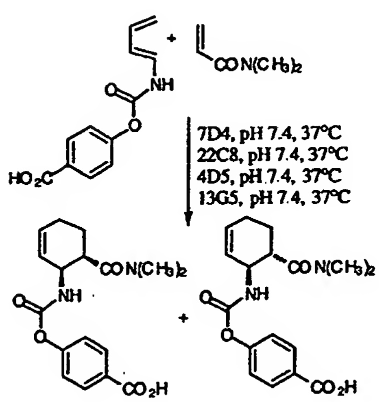
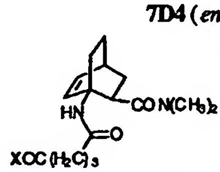
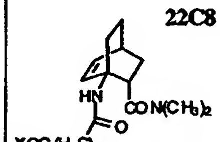
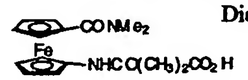
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ µM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 A (1% v/v acetone, 400 µM, amine) B	A: 1.8×10^3 (app.) B: 4.9×10^3 (app.)	A: 1.8×10^{-4} (app.) B: 9.6×10^{-5} (app.)	A: 1.0×10^2 (app.) B: 1.7×10^2 (app.)	16.3

17. Diels–Alder cycloaddition

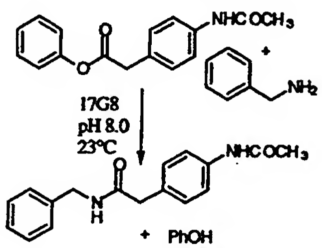
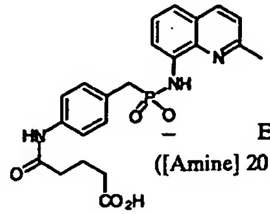
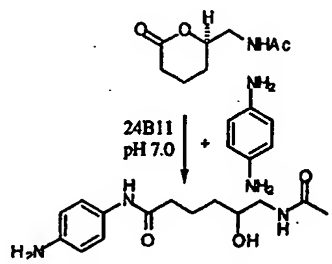
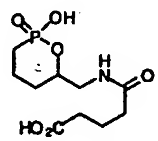
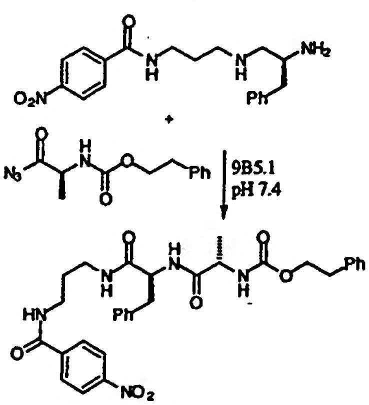
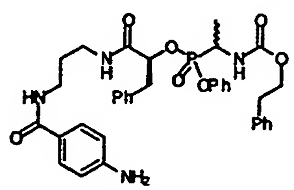
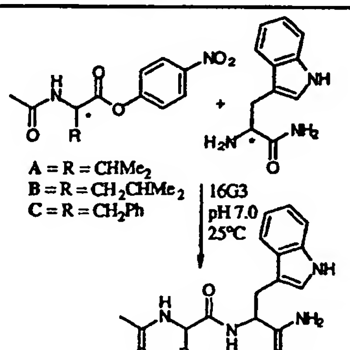
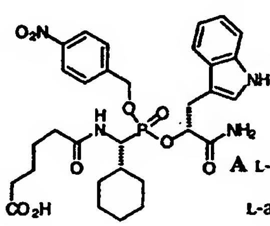
	Dienophile ([Diene] 0.61 mM)  *TSA, Entropic Trap*	2.1×10^4 (app.)	4.3 (app.)	1.1×10^2 (app.)	17.1
	Diene Dienophile  *TSA* K_i 1.3×10^{-1} µM	Diene: 1.1×10^3 Dienophile: 7.4×10^2	4.0×10^1	3.5×10^{-1}	17.2

nr, not reported.

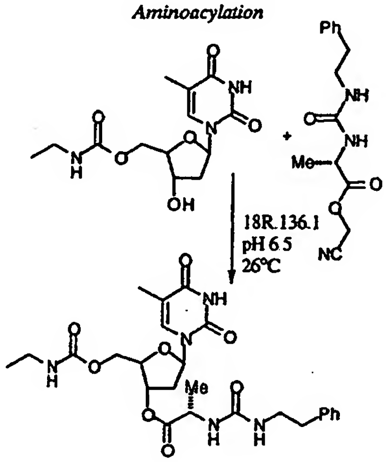
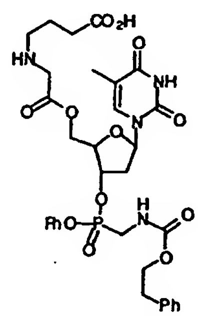
Reaction/conditions	Hapten/comments/K_i		K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 H11, pH 8.0, 18°C	 TSA	Diene	nr	nr	nr	17.3
		Dienophile	8.3×10^3	3.3×10^1	1.8×10^1	
		Esterolysis	1.1×10^3	5.5×10^{-2}	nr	
 309-1G7, pH 7.3; Ar = p-$C_6H_4CONHPr$	 R = $(CH_2)_3CO_2H$; TSA	Dienophile ([*trans*-Diene] 5.0 mM)	3.1×10^3 (app.)	2.0×10^1 (app.)	1.2×10^3 (app.)	17.4
		Dienophile ([*cis*-Diene] 5.0 mM)	3.9×10^3 (app.)	1.1×10^1 (app.)	2.6×10^3 (app.)	
 7D4, pH 7.4, 37°C; 22C8, pH 7.4, 37°C; 4D5, pH 7.4, 37°C; 13G5, pH 7.4, 37°C	**7D4** (*endo*) 	Diene	9.6×10^2	3.4×10^{-3}	4.8	17.5
		Dienophile	1.7×10^3			
	22C8 (*exo*) 	Diene	7.0×10^2	3.2×10^{-3}	1.8×10^1	
		Dienophile	7.5×10^3			
	4D5 (*endo*) 	Diene	1.6×10^3	3.5×10^{-3}	4.9	
		Dienophile	5.9×10^3			
	13G5 (*exo*)	Diene	2.7×10^3	1.2×10^{-3}	6.9×10^1	
		Dienophile	1.0×10^4			
	TSA					

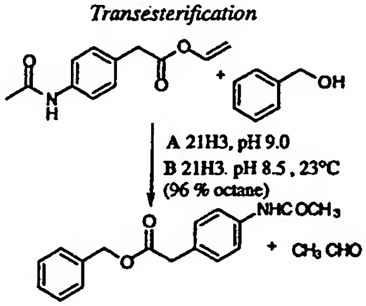
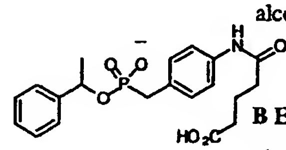
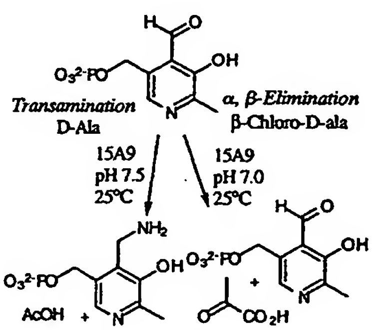
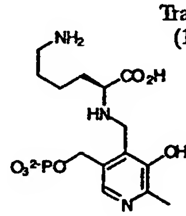
nr, not reported.

18. Acyl transfer reactions

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Amide Formation  17G8 pH 8.0 23°C + PhOH	 Ester ([Amine] 20 μM) *TSA* IC_{50} 1.0×10^1 μM	2.2×10^3 (app.)	2.3×10^{-2} (app.)	1.1×10^1 (app.)	18.1
 24B11 pH 7.0	 Lactone Aniline *TSA* K_i 7.5×10^{-2} μM	4.9×10^3 1.2×10^3	6.6×10^{-2}	1.6×10^1	18.2
Peptide Bond Formation  9B5.1 pH 7.4	Azide Amine  *TSA* K_d 1.9×10^{-2} μM	1.5×10^1 1.5×10^3	5.9×10^{-2}	1.0×10^4	18.3
 A = R = $CHMe_2$ B = R = CH_2CHMe_2 C = R = CH_2Ph 16G3 pH 7.0 25°C	 A L-Ester L-amine *TSA*	4.0×10^3 1.6×10^4	1.3×10^1	1.9×10^2	18.4

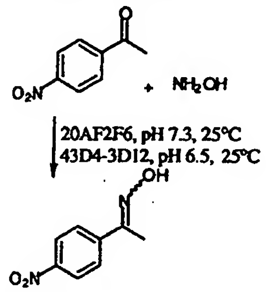
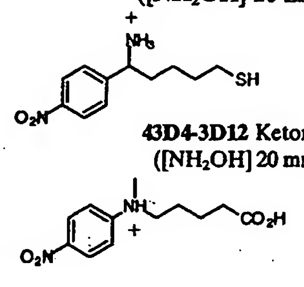
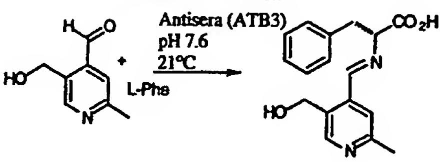
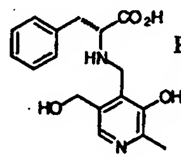
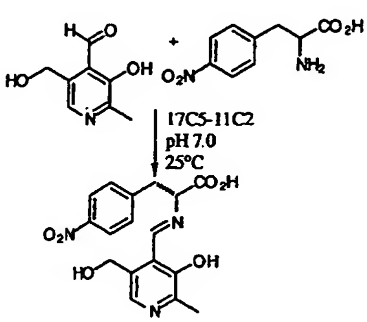
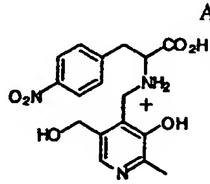
nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Aminoacylation  18R.136.1 pH 6.5 26°C	 Alcohol Ester *TSA* K_d 2.4×10^{-4} μM	7.7×10^2 2.6×10^2	1.4×10^1	5.5×10^4	18.5

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Transesterification  A 21H3, pH 9.0 B 21H3. pH 8.5, 23°C (96 % octane)	A Ester alcohol	3.0×10^3 7.3×10^3	2.1×10^1	nr	18.6
	 B Ester alcohol *TSA* 21H3: K_i (app) 2.0 μM	1.1×10^3 2.3×10^3	3.0	nr	
 *Transamination* D-Ala, 15A9 pH 7.5 25°C; *α, β-Elimination* β-Chloro-D-ala, 15A9 pH 7.0 25°C	Transamination (100 μM PLP)	2.5×10^3 (app.)	4.2×10^{-1} (app.)	nr	18.7
	 Elimination (100 μM PLP)	10×10^3 (app.)	5.0×10^1 (app.)	nr	

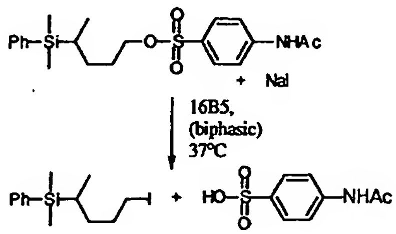
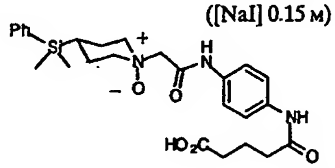
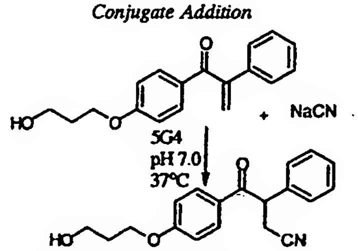
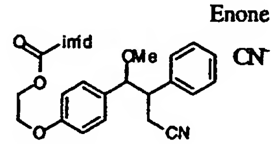
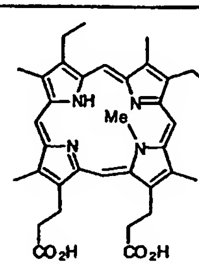
nr, not reported.

19. Animation reactions

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Oxime Formation  20AF2F6: *syn* : *anti* 9:1 43D4-3D12: *syn* : *anti* 1:9	**20A2F6** Ketone ([NH_2OH] 20 mM) **43D4-3D12** Ketone ([NH_2OH] 20 mM)  *TSA*	2.7×10^3 (app.) 9.4×10^2 (app.)	1.1×10^1 (app.) 6.7 (app)	1.7×10^4 (app.) 2.9×10^3 (app.)	**19.1**
Aldimine Formation 	 Pyridoxal L-Phe	3.9×10^3 1.6×10^2	1.5×10^1	nr	**19.2**
	Amino acid Pyridoxal  L-amino acid: K_d 6.0×10^{-3} μM D-amino acid: K_d 1.7×10^{-2} μM	1.2×10^2 7.1×10^2	1.8×10^1	2.1×10^{-1}	**19.3**

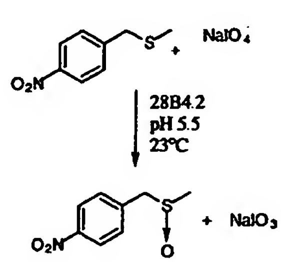
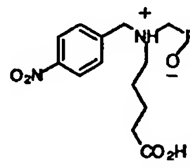
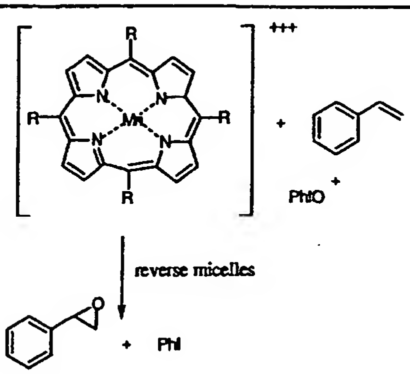
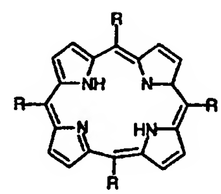
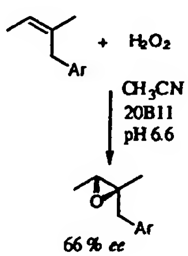
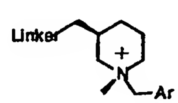
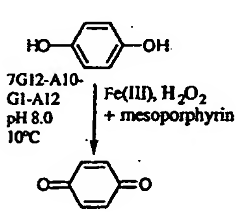
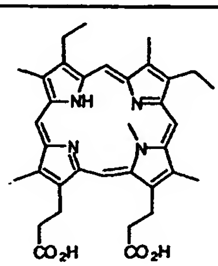
nr, not reported.

20. Miscellaneous

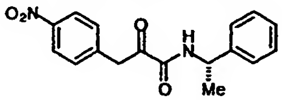
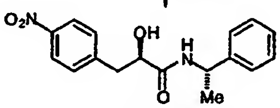
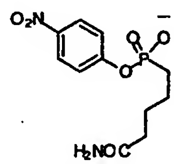
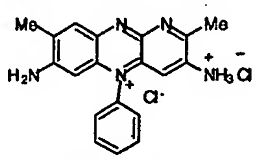
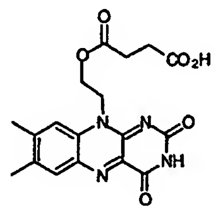
Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
Nucleophilic Substitution  16B5, (biphasic) 37°C	Sulfonate ([NaI] 0.15 M)  NaI ([Sulfonate] 0.75 mM) *TSA* Ki ~ 1.1×10^1 μM	1.3×10^2 (app.) 1.5×10^5 (app.)	2.8×10^{-5} (app.) 2.8×10^{-2} (app.)	5.8×10^2 (app.) 5.8×10^2 (app.)	20.1
Conjugate Addition  + NaCN 5G4 pH 7.0 37°C	Enone CN⁻  *TSA* Ki ~ 1.1×10^1 μM	6.4×10^1 1.4×10^2	2.1×10^{-2}	3.0×10^{-2}	20.2
Porphyrin Metalation Porphyrin + M^{2+} → (7G12-A10-G1-A12, pH 8.0, 26°C) Porphyrin M^{2+}	Zn^{2+} Cu^{2+} 	4.9×10^1 5.0×10^1	5.2×10^{-4} 8.4×10^{-5}	2.6×10^3 1.7×10^3	20.3

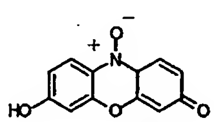
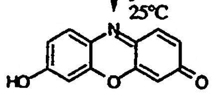
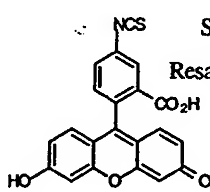
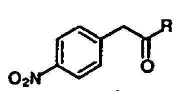
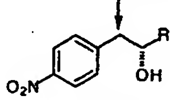
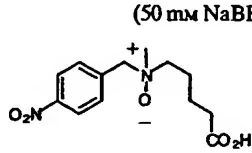
nr, not reported.

21. Oxidations

Reaction/conditions	Hapten/comments/K_i	K_m/ μM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
	 Sulfide NaIO4 *TSA* K_d 5.2×10^{-2} μM	4.3×10^1 2.5×10^2	8.2	9.4×10^6	**21.1**
	 *Metal cofactor complex*	nr	nr	30–60% rate enhance-ment	**21.2**
	 *TSA*	2.6×10^2 (app.)	8.4×10^{-4} (app.)	6.0×10^1 (app.)	**21.3**
	 *Metal cofactor complex*	2.4×10^4	4.0×10^2	2.4×10^1	**21.4**

nr, not reported.

Reaction/conditions	Hapten/comments/K_i	K_m/ µM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 $NaBH_3CN$ (1 mM); A5; pH 5.0; 22°C  *de* > 99 %	 *TSA* K_d 6.1 × 10^{-1} µM	1.2 × 10^3 (app.)	1.0 × 10^{-1} (app.)	2.9 × 10^2 (app.)	22.1
 Safranine T [O] mAb; Flavin; Dithionite Safranine T [R]	 *Cofactor complex* K_d 8.0 × 10^{-3} µM	nr	nr	nr	22.2

Reaction/conditions	Hapten/comments/K_i	K_m/ µM	k_{cat}/ min^{-1}	k_{cat}/ k_{uncat}	Entry
 66D2; pH 5.8; 25°C 	 Sulfite Resazurin	3.0 × 10^3 6.0 × 10^{-1}	1.2	6.0 × 10^1	22.3
 R = Et; R = isopropyl; R = Bn $NaCNBH_3$; 37B39.3; pH 5.0; 22°C  99 % *S* R = Et	R = Et (50 mM $NaBH_3CN$)  $NaBH_3CN$ (0.15 mM R = Et) *TSA* K_d 3.3 × 10^{-2} µM	5.2 × 10^1 (app.) 5.7 × 10^4 (app.)	9.7 × 10^{-2} (app.) 1.7 × 10^{-1} (app.)	k_{uncat} 1.1 × 10^{-3} min^{-1} M^{-1}	22.4

nr, not reported.

HYDROLYTIC AND DISSOCIATIVE PROCESSES

1. *Aliphatic ester hydrolysis*

1.1 (Shen *et al.*, 1992); **1.2** (Tanaka *et al.*, 1996); **1.3** 3B9 (Landry *et al.*, 1993), 15A10 (Yang *et al.*, 1996), polyclonal (Basmadjian *et al.*, 1995); **1.4** (Nakatani *et al.*, 1993); **1.5** (Ikeda *et al.*, 1991); **1.6** (Fujii *et al.*, 1991); **1.7** (Iwabuchi *et al.*, 1994); **1.8** (Miyashita *et al.*, 1993); **1.9** (Kitazume *et al.*, 1994); **1.10** (Campbell *et al.*, 1994); **1.11** (Kitazume *et al.*, 1991a); **1.12** (Kitazume *et al.*, 1991b); **1.13** (Ikeda and Achiwa, 1997); **1.14** Ab (Janda *et al.*, 1989), Ab-I (Janda *et al.*, 1990a); **1.15** (Pollack *et al.*, 1989); **1.16** A (Tawfik *et al.*, 1993), B (Tawfik *et al.*, 1997); **1.17** (Janda *et al.*, 1994); **1.18** (Li *et al.*, 1995b); **1.19** (Izadyar *et al.*, 1993).

2. *Aryl ester hydrolysis*

2.1 (Suga *et al.*, 1994b); **2.2** (Tawfik *et al.*, 1990); **2.3** pH 8.7 (Guo *et al.*, 1994), pH 9.5 (Zhou *et al.*, 1994); **2.4** (Khalaf *et al.*, 1992); **2.5** A (Martin *et al.*, 1991), B (Durfor *et al.*, 1988); **2.6** 30C6 (Janda *et al.*, 1990b), 84A3 (Wade *et al.*, 1993), 27A6 (Janda *et al.*, 1991c); **2.7** KD2-260 (Ohkubo *et al.*, 1993), 7K16.2 (Shokat *et al.*, 1990); **2.8** NPN43C9 (Gibbs *et al.*, 1992a), Fab-1D (Chen *et al.*, 1993); **2.9** (Tramontano *et al.*, 1986); **2.10** (Tramontano *et al.*, 1988); **2.11** (Suga *et al.*, 1994a); **2.12** (Janda *et al.*, 1991b); **2.13** (Napper *et al.*, 1987); **2.14** (Wirsching *et al.*, 1995); **2.15** (Pollack *et al.*, 1988).

3. *Carbonate hydrolysis*

3.1 A (Shokat *et al.*, 1990), B (Jacobs *et al.*, 1987), C (Spitznagel *et al.*, 1993); **3.2** (Pollack *et al.*, 1986); **3.3** PCA270-29 (Gallacher *et al.*, 1991), N-CAT 2-6 (Stahl *et al.*, 1995); **3.4** (Wallace and Iverson, 1996).

4. *Carbamate ester hydrolysis*

4.1 (Van Vranken *et al.*, 1994); **4.2** (Wentworth *et al.*, 1996); **4.3** (Wentworth *et al.*, 1997).

5. *Amide hydrolysis*

5.1 (Martin *et al.*, 1994); **5.2** (Iverson and Lerner, 1989); **5.3** (Janda *et al.*, 1988b); **5.4** (Benedetti *et al.*, 1996); **5.5** (Gallacher *et al.*, 1992); **5.6** (Paul *et al.*, 1989); **5.7** (Li *et al.*, 1995a); **5.8** (Paul *et al.*, 1995).

6. *Phosphate ester hydrolysis*

6.1 (Rosenblum *et al.*, 1995); **6.2** 15C5 (Lavey and Janda, 1996a), 3H5 (Lavey and Janda, 1996b); **6.3** (Brimfield *et al.*, 1993); **6.4** (Weiner *et al.*, 1997); **6.5** (Shuster *et al.*, 1992; Gololobov *et al.*, 1995); **6.6** (Scanlan *et al.*, 1991).

7. *Miscellaneous hydrolyses*

7.1 37C4 (Iverson *et al.*, 1990), polyclonal (Stephens and Iverson, 1993); **7.2** (Yu *et al.*, 1994); **7.3** (Janda *et al.*, 1997); **7.4** A (Reymond *et al.*, 1992, 1993, 1994), B (Reymond *et al.*, 1991), C (Shabat *et al.*, 1995), D (Sinha *et al.*, 1993b), E (Sinha *et al.*, 1993a).

8. *Eliminations*

8.1 (Cravatt *et al.*, 1994); **8.2** 43D4-3D21 (Shokat *et al.*, 1989), 20A2F6 (Uno and Schultz, 1992); **8.3** (Yoon *et al.*, 1996); **8.4** (Thorn *et al.*, 1995); **8.5** (Zhou *et al.*, 1997).

9. *Decarboxylations*

9.1 21D8 (Lewis *et al.*, 1991), 25E10 (Tarasow *et al.*, 1994); **9.2** (Smiley and Benkovic, 1994); **9.3** (Björnestedt *et al.*, 1996); **9.4** (Ashley *et al.*, 1993).

10. *Cycloreversions*

10.1 (Bahr *et al.*, 1996); **10.2** A (Cochran *et al.*, 1988), B (Jacobsen *et al.*, 1995).

11. *Retro-aldol reactions*

11.1 (Flanagan *et al.*, 1996); **11.2** (Reymond, 1995).

INTRAMOLECULAR PROCESSES

12. *Isomerizations*

12.1 (Yli-Kauhaluoma *et al.*, 1996); **12.2** (Jackson and Schultz, 1991); **12.3** (Khettal *et al.*, 1994); **12.4** (Uno *et al.*, 1996).

13. *Rearrangements*

13.1 (Braisted and Schultz, 1994; Ulrich *et al.*, 1996); **13.2** 1F7 (Hilvert *et al.*, 1988; Hilvert and Nared, 1988), 11F1-2E11 (Jackson *et al.*, 1988); **13.3** 2B4 (Gibbs *et al.*, 1992b), RG2-23C7 (Liotta *et al.*, 1993); **13.4** (Chen *et al.*, 1994); **13.5** (Willner *et al.*, 1994).

14. *Epoxide opening*

14.1 1 (Janda *et al.*, 1993; Shevlin *et al.*, 1994), 2 (Janda *et al.*, 1995).

15. *Cationic cyclization*

15.1 TX1-4C6 (Li *et al.*, 1994), TMI 87D7 (Li *et al.*, 1995c); **15.2** (Li *et al.*, 1996); **15.3** (Hasserodt *et al.*, 1996); **15.4** (Hasserodt *et al.*, 1997).

16. *Aldol reactions*

16.1 (Koch *et al.*, 1995).

BIMOLECULAR ASSOCIATIVE AND SUBSTITUTION REACTIONS

16. *Aldol reactions continued*

16.2 (Wagner *et al.*, 1995); **16.3** A (Reymond and Chen, 1995a), B (Reymond and Chen, 1995b; Zhong *et al.*, 1997).

17. *Diels–Alder cycloaddition*

17.1 (Hilvert *et al.*, 1989); **17.2** (Braisted and Schultz, 1990); **17.3** (Suckling *et al.*, 1993); **17.4** *trans* (Meekel *et al.*, 1995), *cis* (Resmini *et al.*, 1996); **17.5** 7D4 and 22C8 (Gouverneur *et al.*, 1993), 4D5 and 13G5 (Yli-Kauhaluoma *et al.*, 1995).

18. *Acyl transfer reactions*

18.1 (Janda *et al.*, 1988a); **18.2** (Benkovic *et al.*, 1988); **18.3** (Jacobsen and Schultz, 1994); **18.4** (Hirschmann *et al.*, 1994; Smithrud *et al.*, 1997); **18.5** (Jacobsen *et al.*, 1992); **18.6** A (Wirsching *et al.*, 1991), B (Ashley and Janda, 1992); **18.7** (Gramatikova and Christen, 1996).

19. *Amination reactions*

19.1 (Uno *et al.*, 1994); **19.2** (Tubul *et al.*, 1994); **19.3** (Cochran *et al.*, 1991).

20. *Miscellaneous*

20.1 (Li *et al.*, 1995d); **20.2** (Cook *et al.*, 1995); **20.3** (Cochran and Schultz, 1990a); for a second example see Kawamura-Konishi *et al.* (1996).

REDOX REACTIONS

21. *Oxidations*

21.1 (Hsieh *et al.*, 1994); **21.2** (Keinan *et al.*, 1990); **21.3** (Koch *et al.*, 1994); **21.4** (Cochran and Schultz, 1990b).

22. *Reductions*

22.1 (Nakayama and Schultz, 1992); **22.2** (Shokat *et al.*, 1988); **22.3** (Janjic and Tramontano, 1989); **22.4** (Hsieh *et al.*, 1993).

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